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# Bioremediation of Petrochemical Hydrocarbons (BTEX) – Review

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ABSTRACT

Worldwide industrial development has released a large number of natural as well as synthetic hazardous compounds into the environment. Petrochemical solid waste is generally associated with more hazardous constituents, and accordingly carries a higher level of public health and environmental risk potential. The petroleum hydrocarbon contaminants from industry can enter the soil, air and water. The current physico-chemical technologies are not sufficient and/or effective to treat the contaminants to the acceptable level as per the Environment Protection Act, 1986. Bioremediation is a promising option for the complete removal and destruction of contaminants. It uses of the living organisms, primarily microorganisms, to degrade or detoxify hazardous wastes into harmless substances such as $CO_2$, water and cell biomass. It is an option that offers the possibility to destroy or generate harmless byproducts using natural biological activity. In recent years, many microbial ecologists have identified various microbial species that are effective degraders of hydrocarbons in natural environments. Microorganisms have been widely used for bioremediation in the form of consortium or single bacterial isolate. The present review describes the toxicity of petroleum monoaromatics (BTEX), their route of entry in the environment and its microbial degradation.

## 1. Introduction

World today where we live in, revolves around energy as regards human growth, biological, physical, economic, industrial and infrastructural growth, science and technology, etc. Petrochemical hydrocarbons have been used as the most prime and multipurpose source of energy apart from the traditional firewood, wind and hydropower. Industrial revolution has led to release of explorations and exploitation of crude oil or petroleum hydrocarbons resulting in tremendous disturbances in the global elemental cycles. Petroleum refining containing natural gas and crude distilleries are used as feed to manufacture a wide variety of petrochemicals. The basic petrochemicals including olefins like ethylene, propylene, butylenes, and butadiene and aromatics like benzene, toluene, and xylenes are manufactured by cracking, reforming, and other processes. Aromatic hydrocarbons are grouped as compounds constituting principally carbon and hydrogen in the form of one or more aromatic rings. Being the second richest type of organics found in nature, they are the mixture of monoaromatic and poly-aromatic hydrocarbons. The extensive discharge of aromatic hydrocarbons through spillages and leakage from underground tanks has caused wide-ranging contamination of surface soils and sea and groundwater environment [1].

Gasoline is the key product in the petrochemical industry and consists of approximately 70% aliphatic linear and branched hydrocarbons, and 30% monoaromatic hydrocarbons, including xylenes, toluene, di- and tri-methylbenzenes, ethylbenzenes and benzene (collectively known as BTEX). These compounds are used as industrial solvents and they supply the preliminary materials for the production of pharmaceuticals, agrochemicals, polymers, explosives and many other day-to-day products. These volatile compounds act as essential solvent in industrial operations. They are let out in the environment during manufacture, transportation, usage and disposal, seepage in the storage tanks and pipelines and through leachate from landfills and categorized among the hazardous compounds by the U.S. Environmental Protection Agency [2]. The maximum permissible levels for monoaromatic compounds in potable water are 0.05, 1, 0.7 and 10 ppm for benzene, toluene, ethylbenzene and isomers of xylenes, respectively [3]. The Clean Air Act Amendments of 1990 (CAAA 90) proposed by the US Environmental Protection Agency (EPA) emphasizes specifically on the management, handling and treatment of BTEX compounds which are among the 188 hazardous air pollutants (HAPs) linked this recognized Act. Ever since these BTEX compounds have been shown to have detrimental impact on environment and human health such as expressing carcinogenic, mutagenic as well as teratogenic effects; they have drawn attention of many researchers. For example; Benzene has been chosen a prime target for estimation of pollution levels in the urban atmosphere being a genotoxic carcinogen, having fatal effect as well as expressing mutagenicity ability [4-8]. There have been a numerous physical and chemical techniques traditionally carried out to remove these toxic compounds from the industrial wastes (Fig. 1). Chemical methods comprise of thermal and catalytic destructions, ozonization and chlorination while the physical include condensation, adsorption on solids or absorption in liquids. However, these techniques are not cost-effective and can result in partial decomposition of contaminants. The ideal solution for pollution abatement is biological degradation of these pollutants aptly known as bioremediation. It is the most efficient and innovative technology that uses biological systems for management of contaminants. It exploits of diversity of microorganisms to detoxify or completely mineralize the pollutants owing to their metabolic capabilities. It has been applied extensively for removal and degradation of many environmental pollutants including the products of petroleum industry. The bioremediation approach can be applied on site (in situ) or off site (ex situ), either mediated by mixed microbial consortia and/or pure microbial strains and plants or even by natural attenuation. Moreover, it encompasses ex-situ engineered methods using bioreactors and enzyme catalysed breakdown. Over past few decades, noteworthy research has been undertaken to study the microbial interactions with the monoaromatic compounds and biodegradation studies have resulted in isolation of numerous bacteria (aerobic and anaerobic), fungi and algae which are capable of degrading low molecular weight aromatic compounds.

The focus of this review is on the occurrence and toxicity of the monoaromatic hydrocarbons mainly BTEX, the ability of bacteria, fungi, algae and plants, including their degradation pathways using these organisms and the available options for bioremediation and approaches for improving microbial degradation.

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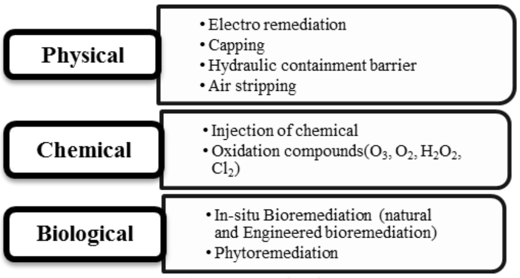
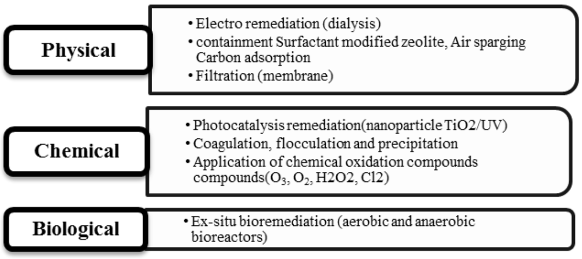


**(a)** In-situ Technologies



**(b)** Ex-situ Technologies

**Fig. 1** Summary of remediation methods available for Monoaromatics [9-16]

## 2. Occurrence and Toxicity Monoaromatic Hydrocarbons (BTEX)

### 2.1 Occurrence in Nature

Monoaromatic BTEX hydrocarbons are commonly found as one of the chief components in gasoline and are generally volatile substances [17]. BTEX compounds may consist of more than 50–60% of the mass that goes into solution when gasoline is mixed with water [18]. They are mobile and remain in contaminated water and effluent as they are not strongly adsorbed [19, 20]. Groundwater gets polluted by monoaromatic compounds due to release of petrol, gasoline, diesel and petrochemical products from storage tanks and wastes from oil industries. Solubility of benzene, toluene, ethyl benzene, xylenes and gasoline in water are respectively 18, 25, 3, 20, 50–100 ppm when gasoline is introduced into water [18]. Percent volume of benzene, toluene, ethylbenzene and xylenes in gasoline, are 1, 1.5, <1–1.5 and 8–10, respectively [21].

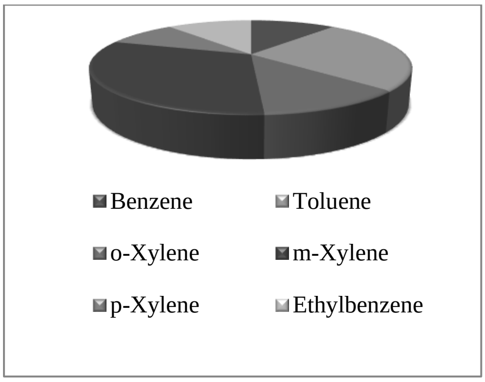


**Fig. 2** Composition of BTEX in Gasoline

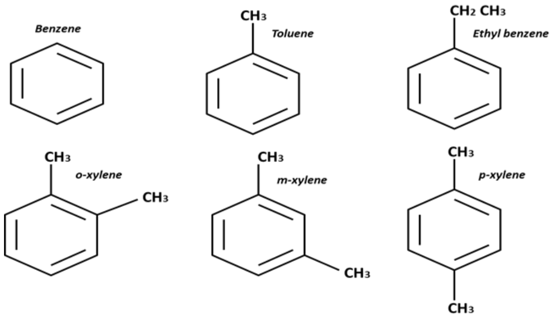


**Fig. 3** Chemical structures of Benzene, Toluene, Ethylbenzene, ortho-xylene, meta-xylene and para-xylene

*Benzene*

Benzene is a colorless liquid that has a sweet odor and is very flammable. Benzene is volatile and readily dissolves in water. Environmental benzene is both natural and anthropogenic in origin. Typically, benzene is used to make other chemicals such as styrene, cumene, and cyclohexane. In industry, benzene is used to produce rubbers, lubricants, dyes, detergents, drugs, and pesticides. Volcanoes and forest fires also contribute to the amount of benzene in the environment. The majority of benzene that is wet deposited to soil or water returns to the air through volatilization. The reaction of photochemically produced hydroxyl radicals with benzene is the most important pathway for the degradation of atmospheric benzene.

*Toluene*

Toluene is a colorless liquid with a sweet, strong odor. Toluene occurs naturally as a component of many petroleum products. It is used as a solvent, particularly in aviation gasoline (Avgas), and in making chemicals, perfumes, medicines, dyes, explosives and detergents. It acts as a solvent for paints, coatings, gums, oils and resins. Along with its uses it also possesses extreme health hazards. It is considered as a teratogen and extremely harmful to human exposure affecting nervous system.

*Ethylbenzene*

It is used mostly in gasoline and aviation fuel additive. It may also be present in consumer products such as paints, inks, plastics and pesticides.

*Xylene*

Xylene is an aromatic hydrocarbon widely used in industry and medical technology as a solvent. It is a colorless, sweet-smelling liquid or gas occurring naturally in petroleum, coal and wood tar, and is so named as it is found in crude wood spirit. Exposure to xylene can occur via inhalation, ingestion, eye or skin contact. It causes health effects from both acute (<14 days) and also persistent (>365days) exposure [3].

The physical and chemical properties of monoaromatic compounds are important characteristics which help in deciding the fate of these chemicals in the environment (Table 1).

**Table 1** Physical and chemical properties and Significance of BTEX compounds

| Parameters | Benzene | Toluene | Ethyl benzene | Xylene (o-, m-, p-) |
|---|---|---|---|---|
| Formula | $C_6H_6$ | $C_6H_5CH_3$ | $C_6H_5CH_2CH_2CH_3$ | $C_6H_4(CH_3)_2$ |
| IUPAC Name | Cyclohexa-1,3,5-triene | Methyl-benzene | Ethylbenzene | Dimethyl-benzenes |
| Other Name | Benzol | Phenyl-methane<br>Toluol<br>Anisen | Ethylbenzol<br>phenylethane<br>alpha-methyltoluene | Xylols |
| CAS no. | 71-43-2 | 1108-88-3 | 100-41-4 | 1330-20-7 |
| Color | Colorless | Colorless | Colorless | Colorless |
| Melting point | 5.5oC | −95 °C | −95 °C | 13 to −47.4 °C |
| Boiling point | 80.1$^{o}$C | 110.8 $^{o}$C | 136.2 $^{o}$C | 139 to 144 $^{o}$C |
| Molar weight | 78.12 | 92.15 | 106.18 | 106.18 |
| Density (g/mL) | 0.8765 | 0.8669 | 0.8670 | 0.8685 |
| Polarity | Non-polar | Non-polar | Non-polar | Non-polar |
| Solubility(mg/L) | 1780 | 500 | 150 | 150 |
| Soil-water partitioning co-efficient | 97 | 242 | 622 | 570 |
| Henry's law constant (25$^{o}$C) {kPa*m$^3$/Mole} | 0.55 | 0.67 | 0.70 | 0.80 |
| Vapor Pressure (mmHg) | 76 | 22 | 7 | 6.5 |
| Log $K_w$ | 2.13 | 2.69 | 3.15 | 3.20 |
| Rank in the Priority list of hazardous substances | 6 | 71 | 99 | 58 |
| Maximum contamination level in drinking water (mg/L) | 0.005 | 1 | 0.7 | 10 |

### *2.2 Exposure and Effects of BTEX*

In metropolitan atmosphere, due to increased global consumption of gasoline, benzene, toluene, ethylbenzene and xylene group of aromatic VOCs constitute upto 60% of non-methane VOCs [19]. This group of compounds acts as a major polluter thus, being an efficient indicator for determining pollution levels of an environment. Due to their relatively high solubility and toxicity, they possess as considerable health threat in contaminated environments. Acute and even short term exposure to these compounds has been associated with skin and sensory irritation, CNS-problems (tiredness, dizziness, headache, loss of coordination) and effects on the respiratory system (eye and nose irritation). Therefore, entry of these compounds in soil and groundwater system, are considered such a serious problem. Ecotoxicity, genotoxicity, carcinogenicity and endocrine interference potentials of the contaminant (s) are considered while determining the risk involved. The reaction of the BTEX with hydroxyl radicals (OH) and/or nitrate ($NO_3$) radicals is found to serve as the dominant degradation processes for aromatic VOCs in the atmosphere and the resulting products add to secondary organic aerosol (SOA) formation by nucleation and condensation [22].

### *2.3 Microbial Degradation of Monoaromatic Hydrocarbons*

Microorganisms have vital role in biological process of remediation. The microorganisms which have the physiological and metabolic capability for pollutant removal are more often bacteria (aerobic as well as anaerobic), but fungi can also play a significant part [23-26]. During bioremediation, catabolic fate of oil pollutants takes place leading to the formation of less toxic materials like $CO_2$., methane, $H_2O$ and inorganic salts [27]. It comprises of two different approaches; *in-situ* and *ex-situ* performed under aerobic or anaerobic conditions. Among all remediation technologies for treating BTEX contaminated groundwater and soil, bioremediation is a low-cost, energy efficient and environmentally sound approach. In general, aerobic biodegradation is considered much faster than anaerobic [28]. In subsurface soil and groundwater environments aerobic microorganisms consume the accessible molecular oxygen faster than it can be replenished. Hence, due to limiting levels of oxygen level, anaerobic degradation of aromatic hydrocarbons may be the determining machinery [29].

## 3. Bacterial Degradation of Monoaromatic Hydrocarbons

It has been known that acclimatized communities, i.e., those which have been previously exposed to hydrocarbons, display higher biodegradation rates than communities with no account of hydrocarbon contamination (Fig. 4). The mechanisms of adaptation include both selective enrichment and genetic modifications, resulting in a net rise in the number of hydrocarbon utilizing organisms and in the pool of hydrocarbon-catabolizing genes within the community. An array of gram positive as well as gram negative bacteria has been accounted to have the capacity to degrade wide range of hydrocarbons as carbon and energy (as seen in Table 2). They transform the substance through metabolic or enzymatic pathways. Biodegradation processes vary largely, but normally the final product of the degradation is carbon dioxide or methane.

**Table 2** Bacterial Species involved in Biodegradation of BTEX

| Bacterial Species | Strains | Aromatic Hydrocarbon | Reference |
|---|---|---|---|
| Single Isolate Used<br>*Pseudomonas putida* | MHF 7109 | Benzene, Toluene, Xylene | [30] |
| *Mycobacterium cosmeticum* | byf-4 | Benzene, Toluene, o-Xylene | [31] |
| *Rhodococcus rhodochrous* | | Benzene, Toluene, Ethylbenzene, Xylene | [32] |
| *Pseudomonas spp.* | ATCC 555 95 | Benzene, Toluene, p-Xylene | [33] |
| *Rhodococcus sp.* | RR1 & RR2 | Benzene, Toluene, m- and p-Xylene | [34] |
| *Pseudomonas putida* | F1 | Benzene, Toluene, Ethylbenzene | [35] |
| *Ralstonia picketti* | PK01 | Toluene | [36] |
| *Burkholderia cepacia* | G4 | Toluene | [37] |
| *Blastochloris sulfoviridia* | ToP1 | Toluene | [4] |
| *Rhocococcus spp.* | DK17 | Benzene, m-xylene, o-xylene | [38] |
| *Achromobacter xylosooxidans* | | Benzene, Toluene, Ethylbenzene, Xylene | [39] |
| *Pseudomonas sp.*<br>*B.stereothermophilus*<br>*Vibrio spp.*<br>*Nocardia spp.*<br>*Corynebacterium spp.*<br>*Alcaligenes spp.* | | Benzene, Toluene, Ethylbenzene, Xylene | [40] |
| *Rhodococcus pyridinovorans* | PYJ-1 | Benzene, Toluene, m -Xylene | [41] |
| Co-metabolism-<br>*Pseudomonas aeruginosa*<br>*Bacillus subtilis* | | Benzene, Toluene, Xylene | [42] |
| *Pseudomonas mendicina* | KR1 | Benzene, Toluene, Ethylbenzene, Xylene | [43] |
| *Janibacter spp.* | SB2 | Toluene | [44] |
| *Paenibacillus pabuli,*<br>*Micromonospora spp.*<br>*Proteus mirabilis*<br>*Bacillus pumili,*<br>*Burkholderia spp.*<br>*Xanthomonas spp.*<br>*Bacillus coagulans,*<br>*Bacillus pallides,*<br>*Bacillus smithii,*<br>*Klebsialla pneumonia* | | Benzene, Toluene, Ethylbenzene, Xylene | [45] |
| *Micrococcus varions* | EPR1S14 | Toluene | [1] |
| *Bacillus subtilis* | EPRS12 | | |
| *Bacillus licheniformis* | EPR1S21 | | |
| *Bacillus laterosporus* | | | |
| *Pseudomonas putida* | EPR1S41 | | |
| *Bacillus firmus* | DAF1 | | |
| *Acientobacter spp.* | EPR1S22<br>EPR1S32 | | |
| *A.sorbia*<br>*B.sterothermophilus*<br>*E.sakazaki*<br>*S.lentus*<br>*P.putida* | | Benzene, Toluene | [46] |
| *Pseudomonas putida* | pWW0 | Toluene, m- and p-Xylene | [47] |
| *Commonas spp.*<br>*Flavobacterium spp.*<br>*Cytophaga spp.*<br>*Xanthomonas spp.*<br>*Nocardia spp.*<br>*Mycobacterium spp.*<br>*Corynebacterium spp.*<br>*Arthrobacter spp.*<br>*Bacillus spp.*<br>*Burkholderia spp.* | | Benzene, Toluene, Xylene | [48] |
| *Burkholderia cepacia* | G4 | | [37] |
| *Pseudomonas paucimobilis* | | Toluene, m- and p-Xylene | [49] |
| *Pseudomonas picketti* | PK01 | Toluene | [50] |
| *Mycobacterium rhodococcus*<br>*Pseudomonas aeruginosa* | | Benzene | [51] |
| *Pseudomonas putida* | 39/D | Toluene, | [52] |
| *Nocardia coralline* | V-49 | Ethylbenzene, Xylene | |
| *Pseudomonas putida* | BP18 | Benzene, Toluene, Ethylbenzene, Xylene | [53] |
| *Pseudomonas putida* | ML2 | Benzene, Toluene, Ethylbenzene, Xylene | [54] |

| | | | |
|---|---|---|---|
| *Sphingomonas spp.* | DSM 6900 | Toluene, m- and p-Xylene | [55] |
| *Pseudomonas spp.* | NCIB 9816-4 | Toluene | [56] |
| *Pseudomonas pseudoalcaligenes* | KF707 | Toluene | [57] |
| Halotolerant bacteria- | | Benzene | [58] |
| *Marinobacter spp.* | | | |
| *Streptomyces spp.* | | | |
| *Arhodomonas app.* | | | |
| *Selenastrun capricomutum* | | Toluene | [59] |
| *Azoarcus tolulyticus* | Tol4 | | |
| *Azoarcus spp.* | EbN1 | | |
| *Magnetospirillum spp.* | | | |
| *Alphaproteobacteria* | | | |
| *Betaproteobacteria* | | | |
| *Aromotoleum aromaticum* | | | |
| *Georgfuchsia toluolica* | | | |
| *Deltaproteobacterium* | | | |
| *Geobacter grbiciae* | | | |
| *Geobacter spp.* | TMJ1 | | |
| *Desulfitobacterium aromaticivorans* | G5G6 | | |
| *Georgfuchsia toluolica* | | | |
| *Desulfolacula toluolica* | GS-15 | | |
| *Geobacter metallireducens* | | | |
| *Desulfotomaculum ovata* | | Xylene | [59] |
| *Betaproteobacteria* | | | |
| *Denitratisoma oestradiolium* | | | |
| *Geobacter spp.* | | | |
| *Peptococcaceae* | | | |
| *Thernincola* | | | |
| *Pelomonas* | | Benzene | [59] |
| *Epsilonproteobacteria* | | | |
| *Clostridia* | | | |
| *Chloroflexi* | | | |
| *Bacterioides* | | | |
| *Sulfurovum* | | | |
| *Cryptanaerobacter* | | | |
| *Methanosaeta-like Archea* | | | |
| *Aromatoluem aromaticum* | DN11 | | |
| *Azoarus spp.* | | | |
| *Zooglea resiniphilia* | PIV-3A 2W | | |
| *Mesorhizobium spp.* | WG | | |
| *Stenotrophomanas acidaminiphilia* | | | |
| *Alicycliphilus denitrificans* | K601 | | |
| *Dechloromonas agitate* | | | |
| *Dechloromonas spp.* | RCB | | |
| *Pseudomonas putida* | CCMI 852 | Benzene, Toluene, Xylene | [60] |
| *Pseudomonas putida* | AY-10 | Benzene, Toluene, Ethylbenzene, Xylene | [61] |
| *Pseudomonas putida* | mt-2 | m-Xylene | [62] |
| *Pseudoxanthomonas spadix* | BD-a59 | Benzene, Toluene, Ethylbenzene, Xylene | [63] |
| *Acinebacter spp.* | B113 | Benzene, Toluene, Ethylbenzene | [64] |
| *Pseudomonas stutzeri* | OX1 | o-Xylene | [65] |
| *Ferroglobus placidus* | | Benzene | [66] |
| *Acinitobacter calcoaceticus* | A2 | Benzene, Toluene, Ethylbenzene, Xylene | [67] |

### *3.1 Aerobic Bacterial Degradation*

Aerobic organisms perform cellular respiration to obtain energy utilizing Aerobic organisms carrying out cellular respiration, utilize oxygen to oxidize substrates (for example sugars and fats) in order to obtain energy. This leads to speedy and absolute degradation of the majority of pollutants. Mixed microbial communities have the most powerful biodegradative potential owing to genetic information of more than one organism is necessary to degrade the complex mixtures of organic compounds present in contaminated areas. The initial attack of aliphatic and cycloaliphatic hydrocarbons require molecular oxygen. Oxidation of hydrocarbons is classified as being terminal or diterminal. The mono-terminal oxidation is the key pathway. These early reactions, viz. hydroxylation and dehydrogenation, are also common to pathways of degradation of other aromatic hydrocarbons. It progresses via the formation of the resultant alcohol, aldehyde, and fatty acid. β-Oxidation of the fatty acids results in the formation of acetyl-CoA. n-Alkanes with an uneven number of carbon atoms are degraded to propionyl-CoA, which is later carboxylated to methyl malonyl-CoA and further converted to succinyl-CoA. Fatty acids of a physiological chain length may be directly incorporate into membrane lipids, but the majority of degradation products are introduced into the tricarboxylic acid (TCA) cycle. Branching present in hydrocarbons reduces the rate of biodegradation. Methyl side groups do not radically decrease the biodegradability, whereas complex branching chains, e.g., the tertiary butyl group, interfere with the action of the degradative enzymes.

The primary step of benzene oxidation is a hydroxylation catalyzed by a dioxygenase (as seen in Fig. 5). The product, a diol, is then converted to catechol by a dehydrogenase. The addition of a substituent group onto the benzene ring renders alternative mechanisms possible to attack side chains or to oxidize the aromatic ring. The versatility of bacteria is based on the existence of catabolic plasmids. Catabolic plasmids have been found to encode enzymes degrading naturally occurring aromatics such as camphor, naphthalene, and salicylate. Most of the catabolic plasmids are self-transmissible and have a wide host range. The majority of gram negative soil bacteria isolated from polluted areas possess degradative plasmids, mainly the so called TOL plasmids. The main reaction involved in the oxidation of toluene is the methyl group hydroxylation. The methyl group of toluene is oxidized gradually to the corresponding alcohol, aldehyde, andcarboxylic group. Benzoate formed or its alkylated derivatives are then oxidized by toluate dioxygenase and decarboxylated to catechol. The oxygenolytic cleavage of the aromatic ring occurs via o- or m-cleavage [48].

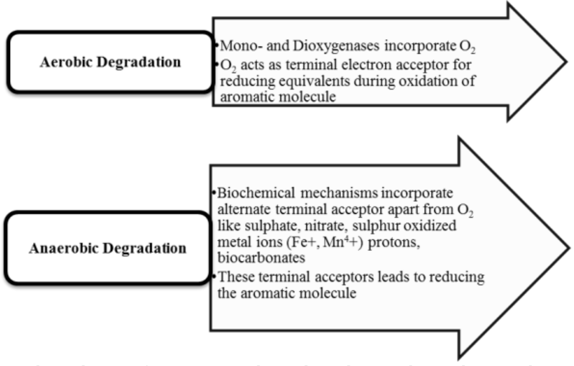


**Fig. 4** Biodegradation of Aromatic Hydrocarbons by Aerobic and Anaerobic process

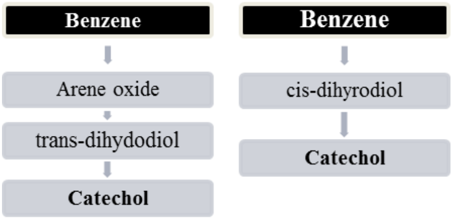


**Fig. 5** Dioxygenase Reaction for Initial Cleavage of Benzene and Catechol formation (i) Initial attack on monoaromatic oxygenases (ii)Monooxygenases incorporate one atom of oxygen of $O_2$ into the substrate, the second atom is reduced to$H_2O$ (iii)Dioxygenases incorporate both atoms into the substrate [48]

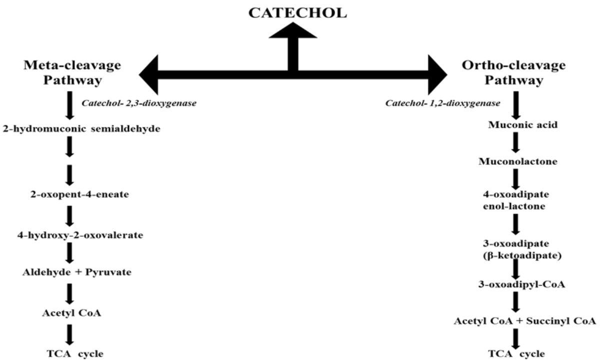


**Fig. 6** Catabolism of Catechol by ortho and meta-cleavage pathways during aerobic process of biodegradation

### *3.2 Anaerobic Bacterial Degradation*

Anaerobic degradation of aromatic hydrocarbons is a multistep process in which microorganisms break down hydrocarbon in the absence of oxygen. Benzene, toluene, xylene and ethylbenzene compounds have been observed to be mineralized under anaerobic conditions (as seen in Table 3) under a wide variety of electron accepting conditions ranging from methanogenic, sulfate reducing, iron reducing, manganese reducing to denitrification [68-71]. Anaerobic BTEX degrading activities and microorganisms are typically associated with sites which have shown to be long term polluted with petroleum hydrocarbons [72]. Anaerobic biodegradation of benzene was found to be relatively slow carried out using laboratory enrichment cultures required almost two years to mineralize benzene under methanogenic conditions [70]. In the similar context, anaerobic toluene degradation is observed to be within as less as 6 hrs using denitrifying bacteria [69].

**Table 3** Biodegradation of BTEX under different redox conditions

| Compound | Aerobic Condition | Denitrifying Condition | Sulfate Reducing Condition | Iron Reducing Condition | Methanogenic Conditions |
|---|---|---|---|---|---|
| Benzene | ++ | - | + | - | + |
| Toluene | ++ | ++ | + | + | + |
| m-xylene | ++ | ++ | + | + | + |
| p-xylene | ++ | ++ | + | + | + |
| o-xylene | ++ | + | + |  | +/- |
| Ethylbenzene | ++ | +/- | - | - | +/- |

**Table 4** Biodegradation under Anoxic (Anerobic conditions)

| Anaerobic Process for Biodegradation under | Compound | Reference |
|---|---|---|
| Nitrate Reduction Condition | Benzene | [73] |
|  | Benzene | [74] |
| Sulphate Reducing Condition | Benzene in Mixtures of BTX | [7] |
|  | Petroleum contaminated harbor sediments | [17] |
|  | Benzene | [75] |
|  | Toluene | [76, 50] |
|  | Toluene | [77] |
|  | Hydrocarbons | [15] |
|  | Toluene, Xylene | [78] |
|  | o-Xylene, m-Xylene and Homologous Alkyl benzene | [79] |
|  | Benzene | [80] |
|  | Toluene | [50] |
| Iron Reducing Conditions | Aromatic Hydrocarbons | [81] |
|  | Benzene | [82] |
|  | Napthalene and Benzene | [22] |
|  | Toluene, Phenol and p-cresol | [83] |
|  | Benzene | [82] |
| Transformation by Methanogens (Cultures and Sediments) | Toluene | [84] |
|  | Toluene and Benzene | [84] |
| Complete Mineralization of Compound in Anoxic conditions | Benzene | [85] |

### *3.3 Biodegradation of Alkyl Substituted Anaerobic Degradation*

Anaerobic biological transformations of aromatic compounds involve initial oxidation or reduction of substituent groups, carbon–carbon cleavage of the substituents from the ring, decarboxylations, the removal of O-methyl, sulfur, nitrogen, or halogens from the ring [69,86]. Generally, these transformations lead to the conversion of varied aromatic compounds to benzoate or its coenzyme A (CoA) thio-ester prior to ring reduction and cleavage. The reduction of benzoyl-CoA represents a considerable energy barrier for anaerobic microorganisms. The thio-group present in coenzyme A substantially lowers the mid-point potential of the first electron transfer [87-89] yet, the reduction of benzoyl-CoA represents a key energy barrier [88] which would overcome by ATP hydrolysis [90]. Several pathways are known for anaerobic cleavage of the benzene ring of aromatic compounds with functional groups such as carboxyl or hydroxyl groups (as seen in Figure 7). The anaerobic metabolism of benzene poses a hurdle due to the large topological resource energy [91].

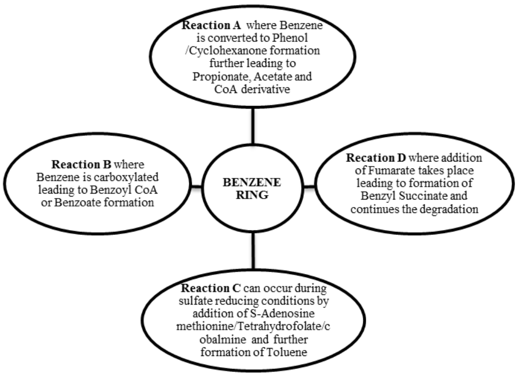


**Fig. 7** Possible mechanisms of the Initial steps of Benzene ring activation during anaerobic conditions (Adapted from [39,63])

### *3.4 Biodegradation of Toluene*

Heider et al. [64] studied microbial biodegradation of Toluene under anaerobic conditions. Toluene was found to react with a fumurate molecule to form a benzylsuccinate molecule. The benzylsuccinate synthase thus formed involves an enzyme radical reaction (presumably a glycine radical). Such reaction has already been documented in denitrifying, sulfate reducing, anoxygenic phototrophic bacteria, and a methanogenic consortium [92]. The pathway proposed is shown in Figure 8, which leads to the formation of benzoyl-CoA, the chief metabolite of anaerobic aromatic compound degradation before the ring saturation and cleavage [93].

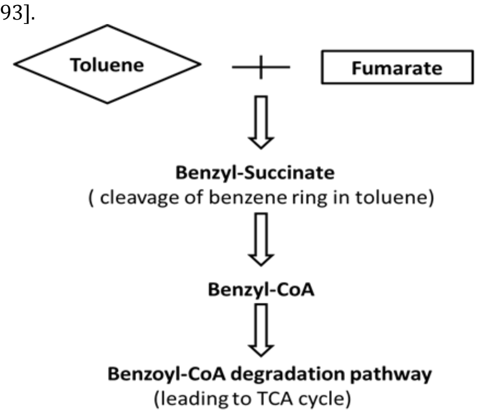


**Fig. 8** Activation of Toluene for anaerobic biodegradation by Fumarate addition (Adapted from [64]

## 4. Bioremediation of Monoaromatics using Fungi

The fungal degradation of aromatic hydrocarbons is regard as one of the efficient methods for removing these pollutants from the environment. One such fungus known for its ability of bioremediation is the white-rot fungus. It contains an unusual enzyme system having a unique group of peroxidases that catalyzes the degradation of the complex plant polymer lignin. This ligninolytic system shows a high level of nonspecificity and oxidizes a very large variety of compounds in addition to lignin. Among these compounds are plentiful environmental pollutants even most of aromatic hydrocarbons. One such white-rot fungus, Phanerochaete chrysosporium, has been studied in great detail with reference to ligninolytic enzymes and the degradation of BTEX compounds [94]. *Cladophialophora sp.* strain T1 (= ATCC MYA-2335), a soil fungus was found to be capable of growth on fraction of gasoline containing all six BTEX components (along with xylene isomers). Alkylated benzenes (toluene, ethylbenzene, and xylenes) were degraded by assimilation and cometabolism. o-xylene and m-xylene were converted to phthalates as end metabolite whereas p-xylene with toluene was seen to be mineralized [14]. The bioremediation was studied using two fungal isolates Paecilomyces variotii and Exophiala oligosperma and were found to utilize toluene as a carbon and energy source and later mineralized it into carbon dioxide [95]. The table 5 given shows varied populations of fungi found to bioremediate BTEX compounds.

**Table 5** Biodegradation of BTEX using Fungi

| Organism | Compound studied for Degradation | Percentage degradation | Metabolites Produced | Reference |
|---|---|---|---|---|
| *Cladophialophora sp.* Strain T1 | BTEX | TE-90%<br>X-60% | Aromatic aldehydes, Alcohols, phenols, Catechols | [14] |
| *Paecilomycetes variotii and Exophialo oligosperma* | T | | Mineralized to $CO_2$ | [95] |
| *Paecilomycetes variotii* | BTEX mixtures | B-45%<br>o-, m-(X)-30% | | |
| *Cladophialophora psammophila* | BTEX | Reduction in concentration | | [96] |
| *Arbuscular Mycorrhizal Fungi G.mosseae, Gi.rosea, Gi.margarita* | BTEX | Residual upto 2-40% | | [97] |
| *Phaenerochete chrysosporium* | BTEX | T-40 to 50%<br>E-98% | Mineralization to $CO_2$ observed | [98] |
| *Trametes versicolor* | BTEX | 71% | | [99] |

## 5. Factors Affecting Bioremediation of Monoaromatics

Various approaches for biodegradation of aromatic hydrocarbons may it be in-situ or ex-situ manifests themselves in their complexity depending on pollutant type and the technological requirements. The effectiveness of the degradation process for the aromatic hydrocarbons depends on the nature of the hydrocarbon contaminant material, environmental conditions and the characteristics of the microbial population that is present. The use of the process of natural attenuation requires no human interaction whereas implementation of accelerated and controlled bioreactor-based process may be directed to exploiting microbial technology and bioprocess engineering to optimize the rates and extents of contaminant degradation. The rate of degradation of BTEXs depends on factors inclusive of physical conditions and the nature, concentration and ratios of various types of monoaromatics present, bioavailability of substrates and properties of the biological system involved.

Hydrocarbon-degrading microbes produce a variety of biosurfactants as part of environment or to comply with increasingly stringent environmental their cell surface or as molecules released extracellularly [100-109]. The efficient selection of biosurfactants may drastically improve the process of degradation [89,110-113]. These biosurfactants and supplementary chemical surfactants enhance removal of petroleum hydrocarbons from soil or solid surfaces. The low-molecular-weight biosurfactants (glycolipids, lipopeptides) are more effective in lowering the interfacial and surface tensions, whereas the high-molecular-weight biosurfactants (like amphipathic polysaccharides, proteins, lipopolysaccharides, and lipoproteins) are effective stabilizers of oil-in-water emulsions [114-116, 104].

Considering the presence of microbial population, the nutrient availability, especially nitrogen and phosphorus, appear the most common limiting factor [117, 107]. The low water solubilities of the majority of petroleum hydrocarbon compounds have the potential to limit the capacity of microbes, which generally exist in aqueous phases, to access and degrade these substrates [30]. It has been observed that bioaugmentation (addition of commercial microbial cultures) is ineffective in aromatic hydrocarbon degradation over biostimulation (nutrient enrichment) of the natural microbial population [118-121, 117, 122,123].

## 6. Enzymes used in Biodegradation of Monoaromatics

The process of bioremediation mainly depends on microorganisms which enzymatically attack the pollutants and convert them to ecologically less harmful or harmless products. Both bacteria and fungi rely on the participation of different intracellular and extracellular enzymes respectively for the remediation of recalcitrant and lignin and organopollutants. In the aerobic conditions, the prime enzymes involved are the monoxygenases. Monooxygenases add in one atom of oxygen into the substrate. They are involved in direct oxidation of the aromatic benzene ring [13]. Monooxygenases are either flavin-dependent monooxygenases or P450 monooxygenases depending on the cofactor present. Monooxygenases act as biocatalysts in bioremediation process and synthetic chemistry due to their highly region selectivity and stereo-selectivity on vast range of substrates. These enzymes need only molecular oxygen for their activities and utilize the substrate as reducing agent. The desulfurization, dehalogenation, denitrification, ammonification, hydroxylation, biotransformation, and biodegradation of various aromatic and aliphatic compounds are catalyzed by monooxygenases. Dioxygenases are the next set of enzymes. They are multicomponent enzyme systems that introduce molecular oxygen into their substrate. Aromatic hydrocarbon dioxygenases, belong to a large family of Rieske non-heme iron oxygenases. These dioxygenases catalyze enantiospecifically the oxygenation of wide range of alkyl substrates formed by the cleavage of aromatic ring. The catechol dioxygenases serve as major component for degrading aromatic molecules in the environment. These leads to formation of catechol intermediates formed either by ortho- or many a times by meta-cleavage pathway deoxygenases. Microbial oxidoreductases help in detoxification of organic compounds in various bacteria and fungi/ and higher plants through oxidative coupling. Microbes take in energy through energy yielding biochemical reactions mediated by these enzymes to cleave chemical bonds and to support the transfer of electrons from a reduced organic substrate (donor) to another chemical compound (acceptor). Also, microbial peroxidases are ubiquitous enzymes that catalyze the oxidation of lignin and other phenolic compounds at the cost of hydrogen peroxide ($H_2O_2$) in the presence of a mediator. However, under anaerobic conditions, benzene degradation can include initial carboxylation, hydrozylation, methylation or reduction of aromatic ring to form benzyl CoA as seen in Fig. 7.

## 7. Bioreactor Based Approaches for Biodegradation

Ex-situ biodegradation of the pollutants is assisted by use of bioreactors under controlled environmental parameters that regulate the degradation process. Slurry phase bioreactors and the attached growth (biofilm) based bioreactors have been widely used to degrade monoaromatic compounds. Slurry growth bioreactors provides improved mass transfer rates, enhanced pollutant degradation and better control and optimization of several environmental parameters [124]. Though suspended growth bioreactors exhibit high removal efficiencies [125-126], their performance have been hindered by surplus biomass accumulation (sludge problems) with continuous operations and substrate inhibition at high pollutant loadings. Another approach to reduce substrate toxicity observed in slurry bioreactors is by the use of an adsorptive medium, growing the biomass on support matrix (biofilm or immobilized cell bioreactors) or the use of a subsequent immiscible phase for a cell based controlled delivery of substrates into the aqueous phase (two-phase partitioning bioreactors like Liquid-Liquid and Solid-Liquid Partitioning Bioreactors). In biofilm based reactors, biomass concentration is developed and maintained on as support matrix thus enhancing metabolic activity and lowering substrate toxicity [18, 127-128].

### *7.1 Case Studies*

### *7.1.1 Bioremediation of Various Monoaromatics in the Two Phase Partitioning Bioreactor*

Two phase partitioning Bioreactors use the water-immiscible and biocompatible organic solvents which float on the surface of cell-containing aqueous phase. This solvent is used to dissolve large concentrations of aromatic hydrocarbons that partition into the aqueous phase at low levels. A very high amount of toxic organic substrates can be added to a bioreactor, the cells experience only low (sub-inhibitory) concentrations. This concept can be readily applied for the treatment of VOCs particularly the BTEX. The degradation of benzene has been efficiently carried out using such liquid-liquid partitioning bioreactor as much as 5000mg/L of the compound was found to be degraded forming intermediates like catechol and 2-hydroxymuconic semialdehyde [109]. Another continuous bioreactor system combining a bubble column (absorption section) and a two-phase bioreactor (degradation section) has been devised to treat a gas stream containing benzene. The bubble column contained hexadecane as an absorbent for benzene, and was systemically chosen considering physical, biological, environmental, operational, and economic factors. The aqueous phase contained *Alcaligenes xylosoxidans* Y234 showing degradative potential for benzene. The degradation of benzene concentration of 120 L/hr and 4.2 mg/L, respectively, with removal efficiency being upto 75% was observed at steady state [129].

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### 7.1.2 Bioremediation of BTEX using Fluidized Bed Reactor

In Fluidized Bed Reactors, particles move up and down within the bed in the well-defined zone of the reactor which has been used for more than two decades for treating industrial wastewater. A co-culture of Pseudomonas putida and Pseudomonas fluorescens immobilized in a fibrous-bed bioreactor was studied to degrade benzene, toluene, ethylbenzene and xylenes (collectively known as BTEX), as sole carbon sources in contaminated water. The kinetics of BTEX biodegradation in the fibrous-bed bioreactor operated under the liquid-continuous condition was studied. BTEX degradation rates upto 600-1000 mg/L/hr were studied. Individual BTEX compounds were also found to be effectively degraded at a retention time of less than 15 hr [130]. The good performance of the fibrous-bed bioreactor is generally attributed to the high cell density and unique cell immobilization process provided by the fibrous matrix used which also allow the use of the reactor for continued regeneration, adaptation and selection of hydrocarbon degraders in the bioreactor environment.

### 7.1.3 GAC-Based Reactors for Bioremediation of Monoaromatics

Packed Bed Reactors are different types of reactors as they are packed with suitable support material like Granulated Activated Carbon (GAC) followed by inoculation with the culture to form a biofilm. GAC adsorption has been used for the treatment of volatile organic compounds. Xing and Hickey [16], studied BTX removal developing a GAC-FBR system by pairing the high-rate biological fluidized bed process (FBR) with granular activated carbon (GAC) adsorption by using the GAC as the biological support surface. The removal has been found to be an efficient method for treatment with up to 99% BTX removal ability.

### 7.1.4 Air Lift Bioreactors

Airlift reactors comprise of two concentric tubes, a riser (inner tube) and a down comer (outer tube) and mixing is accomplished by circulating air at the bottom of the reactor. Due to the force applied by the air at the bottom, liquid in the inner tube moves up thus overflowing (the inner tube) downward and the liquid gets mixed by the eddies current. The use of the partitioning technology in such an airlift bioreactor assembly offers a novel technology that lowers energy intensity in comparison to conventional stirred tank design. The second phase could be solid like the silicon beads or liquid phase like the silicon oil was used. One such use of airlift partitioning bioreactor for the treatment of gases contaminated with a BTEX compounds has been studied. The overall removal efficiencies for the airlift partitioning reactor was seen to be more than 75%, whereas the single-phase airlift showed removal efficiency upto 47.1% [131]. Yet another immobilized cell airlift bioreactor developed for the aerobic bioremediation of simulated diesel fuel contaminated groundwater and analyzed with *p*-xylene and naphthalene in batch and continuous system. The microbial consortia was developed and adapted in the initial reactor setup and the same developed inoculum was then added to the second phase. The varied compound concentrations of *p*-xylene (15, 40 and 77 mg/L) and naphthalene (8, 15 and 22 mg/L) were treated at high degradation rates [132].

### 7.1.5 Membrane Bioreactor System

The use of biofilters, bioscrubbers and air/liquid membranes for treatment of vapor phase waste streams has seen to be gaining importance due to increased regulatory limitations on industrial liberation of atmospheric volatile organic compounds [133]. One system used a commercial membrane unit containing microporous polypropylene fibers while the other used a nonporous silicone tubing membrane module for the delivery of substrate (a combination of benzene, ethylbenzene, toluene, and xylenes) and electron acceptor (O2). The BTEX removal efficiencies were observed to be steadily improved for in the silicone membrane system against the microporous system [134].

### 7.1.6 Anaerobic Bioreactor

The anaerobic processes have a number of advantages over aerobic process of contaminant remediation including, high capability to deteriorate concentrated organic pollutants, lower production of sludge, low energy utilization and biogas (methane) production which is considered as energy resource. A horizontal-flow anaerobic immobilized biomass (HAIB) reactor has been developed under denitrifying conditions by filling the two HAIB reactors with polyurethane foam matrices for biomass attachment. The reactor is fed with synthetic substrate containing protein, carbohydrates, sodium bicarbonate and BTEX solution in ethanol, at an definite Hydraulic retention time (HRT), the hydrocarbon removal efficiencies of 99 % at the varying initial concentrations: benzene 6.7 mg/L, toluene 4.9 mg/L, m-xylene and p-xylene 7.2 mg/L and ethylbenzene 3.7 mg/L. When the reactor was fed with gasoline-contaminated water it showed hydrocarbon removal efficiencies of 96 % at the concentrations: benzene, 4.9 mg/L; toluene, 7.2 mg/L and m-xylene, 3.7 mg/L. The microbial characterization showed the predominance of denitrifying microbial metabolism in the initial segment of reactor whereas from the first sampling port onwards the methanogenic metabolism gradually dominated [135]. Such anaerobic systems can be profitably used for treatment of monoaromatic hydrocarbons under different anoxic conditions for higher levels of degradation. Similarly, toluene and xylene were found to be degraded in an anaerobic column bioreactor. A biofilm of *Pseudomonas putida* (PTCC 1694) was used as a support system and reactor system was found to considerably decrease chemical oxygen demand (COD) of aromatic influent and individual degradation for xylene and toluene were found to be approximately 62 and 65%, respectively. As high uptake concentrations as 7000 and 10000 mg xylene and toluene per liter for Pseudomonas putida were studied showing the COD removal efficiencies of 74 and 80% for 7000 mg/l xylene and toluene respectively [136].

### 7.1.7 Phytoremediation

Phytoremediation is a relatively novel technique to clean contaminated soils using plant as a source for remediation. Plants influence the water balance of a site; they alter redox potential and pH, and encourage microbial activity of the soil. Such indirect influences may hasten degradation in the root zone or reduce leaching of compounds to groundwater. The compounds taken up into plants may be metabolized, accumulated, or volatilized into air. Based on these processes, numerous phytoremediation methods have been developed: Phytoextraction, rhizofiltration, phytostabilisation, rhizospheric and phytodegradation, pump and tree, land farming, phytovolatilisation, hydraulic control and more. The bioaccumulation experiments showed that the Canna (Canna×generalis) could accumulate BTEX (benzene, toluene, ethylbenzene and xylenes) from root zone and rhizome zone soil and then transport them to the shoot. On individual assessment with these BTEX compounds showed that the sequences for accumulation in the root, rhizome and shoot was predominantly related to their physicochemical properties. As for the removal efficiency, the Canna could remove about 80% of BTEX in the root zone and rhizome zone soil in 21 days. Additionally, the removal efficiency in BTEX contaminated soil with 40% water content was slightly higher than that observed with 20% soil water content [137]. The biodegradation capabilities of poplar and its associated rhizospheric and endophytic microorganisms for treating BTEX contaminated groundwater plume were studied. Around 275 poplar trees planted on a field site near a car factory for bioscreening. The rigorous monitoring for a span of 6 long years after the planting of trees was carried out which indicated that the once the poplar tree roots had reached the contaminated groundwater zone, the poplar trees and their associated microorganisms played an active role in the remediation of the BTEX plume, resulting in complete control of the contamination. The examination of the microbial communities associated with poplar revealed that, once the poplar roots got in contact with the BTEX contaminated groundwater, enrichment occurred of both rhizosphere and endophytic bacteria that were able to degrade toluene. It was found that on BTEX remediation, the numbers of toluene degrading rhizosphere and endophytic bacteria reduced below the detection limit, indicating that their population resulted from selective enrichment by the presence of these pollutants [138].

## 7.2 Recent Advances and Various Approaches for Bioremediation of BTEX Compounds

### 7.2.1 Use of Bacterial Biosensors

Biosensors are systems offering great sensitivity and selectivity for the detection and quantification of target compound that are used to determine the interaction of specific compounds through highly sensitive biorecognition processes. The biological recognition element in such biosensors could be enzymes, antibodies, tissues, or living microbes. Whole-cell biosensors, created by combining a reporter gene to a promoter element stimulated by the target compound, provide the ability to characterize, identify, quantify, and determine the biodegradability of specific pollutants present in a complex mixture with no pretreatment of the environmental samples. A plasmid vector containing the genetic information is inserted into the bacterial strain for the replication of engineered fusion along with the cell's own DNA. The use of biosensors have shown to be more efficient and sensitive over the conventional methods for the continuous monitoring of concentration, transformation, and toxicity of common soil and groundwater pollutants especially the petroleum contaminants such as BTEX. Bundy et al. [139] and Bhattacharyya et al. [90] have designed and developed lux-based bacterial biosensor technologies for examining bioremediation of hydrocarbon-contaminated soils. Depending upon the selected biosensor, they may be used to assess the presence of a particular analyte (specifically induced biosensor) or the responsible pollutant load (constitutively expressed biosensor shown by Bundy et al. 2002 [139], coupled with other main soil biological parameters [140-141]. The toxicity of BTEX compounds and degradation byproducts can also be determined by the use of biosensors selected for testing. For developing such bioreactors understanding and knowledge of the biodegradation metabolism and the individual compound toxicity is essential. A tod-luxCDABE fusion was constructed and introduced into the chromosome of Pseudomonas putida F1, yielding the strain TVA8. The strain was studied to determine the induction of the tod operon when exposed to benzene, toluene, ethylbenzene, and xylene (BTEX) compounds and aqueous solutions of JP-4 jet fuel components. Since the entire system constituted the entire lux cassette (luxCDABE), bacterial bioluminescence was estimated in whole cells without added aldehyde substrate. An increasing response for toluene and a significant response for benzene, m- and p-xylenes were observed [142]. For the detection of BTEX in aqueous solutions, GFP-based Pseudomonas fluorescens strain A506 biosensor was developed [143]. With the advancement in nanotechnology applications an efficient and sensitive tool with versatile operational makeup can be developed for these volatile compounds.

### *7.2.2 Molecular Approaches for Bioremediation*

Bioremediation strategies successfully developed for one particular location might not work for the other. Also, the microbial processes studied for the remediation of pollutants in laboratory controlled environment might not be operated in the field natural environment. The cause of such failures is not easily understandable and many researchers are therefore skeptical to take up the risk of bioremediation procedures for remediation. An additional reason limiting the application of bioremediation is that, contrasting to the concepts of excavation and disposal, which are simple to workout, the metabolism controlling the growth and activity of microorganisms in contaminated environments are complicated and difficult to study even for most learned microbiologists. Bioremediation strategies needs to be designed based on the understanding of the metabolism of microorganisms present in the contaminants, their ability to biodegrade and the response to the changes in the environmental conditions. The introduction of high-throughput methods for DNA sequencing and the study of gene expression and function, also the advancement in assessing the modeling of microbial metabolism are the rational ways to study the bioremediation mechanisms developed in recent past. Like the use of genetic monitoring techniques for the detection of specific nucleic acid sequence and nucleic acid hybridization using specific probes for biodegradative sequences or gene message [64,70,120,52,62]; also DNA hybridization/ PCR-based detection methods for nucleic acid fragment recognition or DNA fingerprints [144-145]; employing Realtime PCR with gene probes with fluorescent dual labeled probe detection tool and primers for identifying specific genotypes encoding the keys steps in BTEX biodegradation and their messenger RNAs [146,43,147-149]. The approach of use of microarray based- environmental studies depending on the various types of probes for phylogenetic oligonucleotides, functional genes, and even studying community genome arrays.

### *7.2.3 Use of 16SrRNA Approach*

A noteworthy advancement in the field of microbial ecology was the discovery of the sequences of highly conserved genes found in all microorganisms, especially the 16S rRNA genes, which could offer phylogenetic characterization of the individual microorganisms that comprise microbial communities. The application of the 16S rRNA approach for bioremediation of BTEX compounds could be for analyzing microbial population at genome level and developing solutions by operating genes to initiate effective degradation mechanism. An increased emphasis on quantifying the levels of mRNA for key bioremediation genes have been put forth. Highly sensitive procedures that can detect mRNA for key bioremediation genes in single cells are made available as increased mRNA concentrations have been known to be associated with faster rates of contaminant degradation. Analysis of the mRNA concentrations for genes apart from those directly engaged in bioremediation might give way to additional insights into the parameters that determine the rate and extent of bioremediation. Assessment of the metabolic status of bioremediating microorganisms at each stage by the analysis of the mRNA concentrations for important genes that are related to responding to stress conditions could aid to discover alterations to contaminated environments that might stimulate bioremediation.

### *7.2.4 In silico Biology*

In order to foresee the performance of an organism in a more complex environment as in the natural habitat (niche), it is essential to have a more detailed view of metabolism in models that can give an insight the outcome of the thousands of individual reactions that are real-time taking place in a microbial cell. The in-silico models of cell metabolism can facilitate solve the problem as it includes the probable metabolic reactions that can be possibly be carried out in an organism, as calculated from the annotated genome, and later with varied thermodynamic possibilities which balances the fluxes of metabolic inputs and outputs of all these reactions are determined to illustrate the entire pathway and output of cell metabolism for a known set of environmental parameters. This provides a steady-state prediction of the limitations of the metabolic networks of a particular cell. This complete approach to understand microbial physiology enables to predict the metabolic reactions of organism to various environmental conditions devoid of the requirement for the actual parameters to be undertaken for each and every reactions taking place in the metabolic pathway.

### *7.2.5 Metagenomics Approach*

Indigenous microbial communities are elementary components of ecosystems, playing vital roles in the metabolism of organic compounds. They are primarily involved in detoxification of polluted sites and adaptability of the organisms in degrading wide range of organic contaminants. Metagenomics is one of the key methods designed to gain knowledge and access to the physiology and genetic foundation of uncultured organisms. Thus, microbial genomics, has come across as a dominant centerpiece helping in direct isolation of genomic DNA from an environment, it circumvents avoid actual culturing the organisms under study, and further cloning of it into a culturable organism confines it for study and maintenance. Community type microarray can be created using whole genomic or even large fragments of genomic DNA inventing from metagenomic libraries [150]. A site highly contaminated with aliphatic and aromatic hydrocarbons was characterized for its extradiol dioxygenase diversity and was examined by functional screening of a fosmid library in *Escherichia coli* with catechol as substrate. The 235 positive clones from inserts of DNA extracted from contaminated soil were comparable to one extradiol dioxygenase encoding gene per 3.6 Mb of DNA screened, demonstrating a strong selection for genes encoding this function. Three subfamilies were identified as being principally present, with 72, 55 and 43 fosmid inserts carrying genes associated to those encoding TbuE of *Ralstonia pickettii* PK01 (EXDO-D), IpbC of *Pseudomonas sp.* JR1 (EXDOK2) or DbtC of *Burkholderia sp.* DBT1 (EXDO-Dbt), respectively, whereas genes encoding enzymes related to XylE of *Pseudomonas putida* mt-2 were not found. Genes encoding oxygenases related to isopropylbenzene dioxygenases were generally observed to be co-localized with genes encoding EXDO-K2 dioxygenases [151].

## 8. Conclusion

Petrochemical industrial waste and their disposal problems has always been an area of concern. The aromatic hydrocarbons may it be monoaromatic or polyaromatic, has a widespread use in petrochemical industry and also has many applications which leads to their accumulation in the environment making them priority pollutants due to their ecotoxicity and health hazards. The conventional methods for their treatment have restrictions like low versatility, assembly of toxic byproducts with high cost. Over last few decades, biological cleanup tools like bioremediation have been explored for the remediation of these contaminants. The present literature provides us a profound understanding of the occurrence and the toxicity levels of especially the monoaromatic compounds BTEX and noteworthy research carried out in the remediation of the compounds. Microorganisms have been an important tool in the biodegradation approaches may it be in-situ or ex-situ methods. Aerobic-anaerobic catabolic pathways have been studied in bacterial and fungal populations and also phytoremediation studies with plants have also been carried out. The better understanding of the biochemical pathways and various bioreactor development applications along with the molecular approaches particularly the use of biosensors and in-silico methods that have been carried out are summarized in this review. This would assist to reveal the possible mechanisms of bioremediation of these hazardous compounds.

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