

2.1 Introduction

Resource-intensive human activities such as mining, crude oil extraction, natural gas production, etc. have although modernized the present society and made human lives more comfortable, the environmental pollution has increased by several folds. Major wastes generated from these activities include BTEX (Benzene, Toluene, Ethylbenzene and Xylene) compounds, phenolic compounds, heavy metals, alkalis, oils, dyes, etc. (El-Naas et al., 2014a; Huang and Li, 2014; Mathur and Balomajumder, 2013; Xin et al., 2013; Zhang et al., 2013). These compounds are classified as hazardous wastes. These are carcinogenic and have adverse impacts on human health and environment (Kim et al., 2014; Stasik et al., 2015). Out of more than five million sites identified as contaminated, about 67% sites are contaminated with petrochemical compounds (CRC CARE, 2013/2014). In addition, recent studies have shown that BTEX and other petrochemical compounds, which are detrimental to human health, are also present in abundance in urban ambient air (Caselli et al., 2010), and therefore require urgent remediation.

Among petrochemical compounds, the emission of carcinogenic volatile organic compounds (VOCs) has become a nightmare for environmentalists and chemical engineers. Their long time exposure causes ailments such as acute and chronic respiratory problems, neurological toxicity, lung cancer, fatigue, headaches, depression, etc. (Brodzik et al., 2014; Chen et al., 2016; Chen et al., 2015b;

Gałęzowska et al., 2016; Huang et al., 2014; Li et al., 2016b; Madureira et al., 2016; Saeaw and Thepanondh 2015). Among VOCs, most serious threats are posed by BTEX compounds (Yaws 1991) which have a plethora of polluting agents (Truc and Oanh 2007). Studies have shown that benzene is the most toxic of all the petrochemical pollutants and has negative health impacts such as leukemia, liver cancer, damage to bone marrow, necrosis, edema, hemorrhage, fibrosis and so on (Bahadar et al., 2014; Bird et al., 2005; Edokpolo et al., 2015; Hosseinzadeh and Moosavi-Movahedi 2016; Melo et al., 2007; Wen et al., 2016;). Adverse effects of hydrocarbon pollutants on health of living beings have been studied by many researchers which are presented in more detail in the following paragraphs.

2.2 Studies on the degradation of petrochemical wastes

Numerous aerobic and anaerobic microorganisms have potential to degrade the petrochemical waste. However, to overcome the involved limitations and effectively degrade all the hydrocarbons and related contaminants, there is a need to develop advanced bioremediation strategies. Figure 2.1 depicts some of the strategies which can be employed to improve the process of bioremediation of contaminated environments. Development of hybrid pathways through genetic manipulation of microorganisms is one of the promising techniques which would drastically improve the process of bioremediation. This would help to produce microorganisms which can deal with multiple contaminants and would result in both horizontal and vertical

evolution of catabolic pathways. Horizontal evolution refers to the expansion of catabolic pathways by using enzymes with wide substrate specificity. However, the vertical evolution refers to the expansion of the range of substrates a microorganism can degrade by utilizing enzymes which channel different chemicals into central catabolic pathways. Current efforts to construct such hybrid pathways have mostly been restricted, mainly due to the limited number of enzymes which are available for use, among other factors (Ramos et al., 1987).

Bioremediation and phytoremediation are often considered to be independent approaches. However, the interactions between microbes and plants can be exploited to effectively degrade the pollutants (Kuiper et al., 2004; Ramos et al., 2009; Ronchel et al., 2001; Wu et al., 2006). Chlorinated organic compounds such as trichloroethylene can be removed by using trees colonized by endophytes (Liu et al., 2007; Ye et al., 1998). It has been shown that it is possible to isolate sphingomonads which are known to colonize plant roots and use them to degrade lindane (Boltner et al., 2007). One of the untouched areas in bioremediation is the engineering of anaerobic degradation pathways into suitable hosts. This would help us to obtain newer strains which would be capable of degrading various recalcitrant compounds. These can be some of the major breakthroughs in the field of bioremediation in the near future.

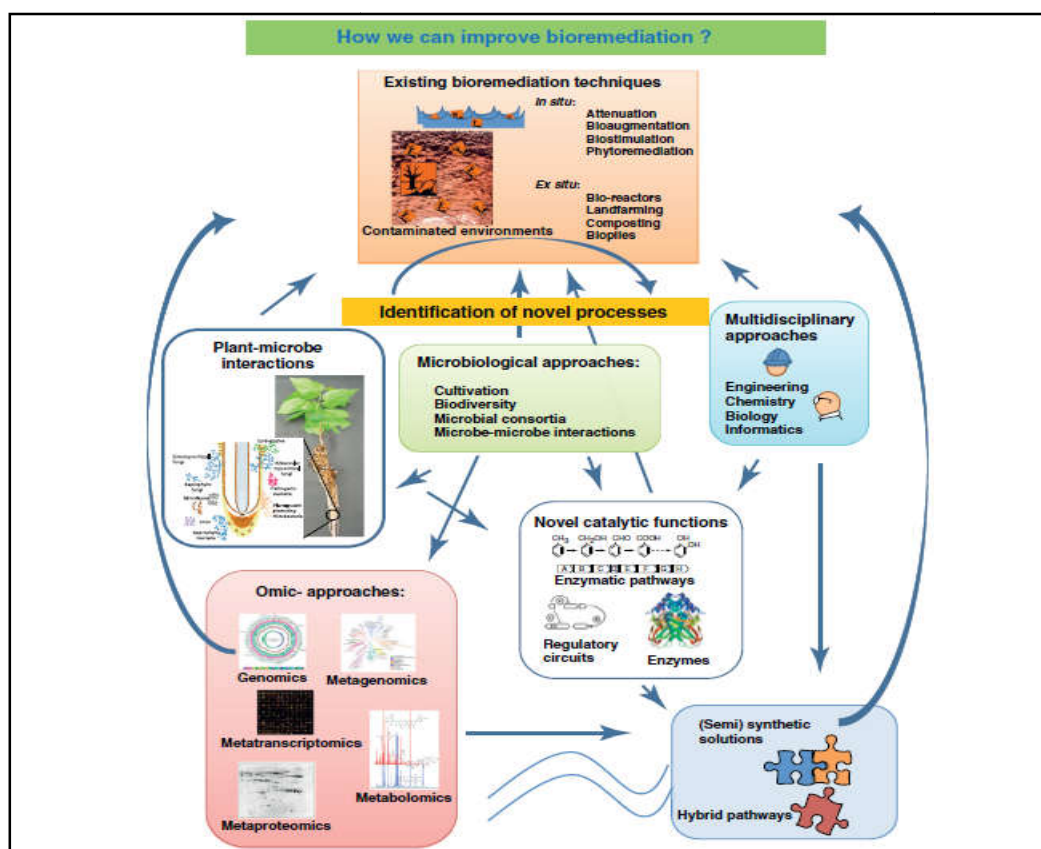


Figure 2.1. A schematic diagram of newer perspectives possible in the realm of bioremediation (adapted from Ramos et al., 2011)

2.3 Adverse effects of petrochemical waste

Occupational or non-occupational exposure to Polycyclic Aromatic Hydrocarbons (PAHs) (e.g. BTEX and other VOCs compounds) is extremely harmful for living organisms, including human beings (Abdel-Shafy and Mansour 2016; Bahadar et al., 2014; Barhoumi et al., 2016; Edokpolo et al., 2015; Fan et al., 2014; Park and Park, 2010; Poli et al., 2016). Mazzeo et al., (2010) reported the cellular damages in the *Allium cepa* test system caused by BTEX. Chen et al., (2008) reported various types of DNA damages in human lymphocytes on being exposed to

hydrocarbons like BTEX and Methyl tert-butyl ether (MTBE). Pariselli et al., (2009) studied the effect of toluene and benzene in air on human lung cells and found variable effects of both the pollutants. Sole toluene (in concentration of 0.25 ppm) causes DNA damage to cells which get repaired within 24 hours post treatment (Pariselli et al., 2009). However, sole benzene did not induce DNA damage, even under different conditions, but it lead to decrease in the glutathione ratio. The presence of benzene and toluene in mixture causes irreparable DNA damage (Wen et al., 2016). Toluene is reported to cause genetic damage and oxidative stress (Kodavanti et al., 2011; Kodavanti et al., 2015; Moro et al., 2012; Singh et al., 2009).

PAH compounds are genotoxic to human as well as plant cells. Genotoxicity is the property of chemical agents that damages the genetic information within a cell causing mutations, which may lead to cancer (Albuquerque et al., 2016; Benford et al., 2010; Borska et al., 2010; Goswami et al., 2016; Kim et al., 2013; Li et al., 2016a; Nakata et al., 2014; Oliveira et al., 2016; Wang et al., 2015). Singh et al., (2007) observed increased apoptotic markers and genotoxicity in a concentration and time-dependent manner in organisms exposed to benzene, toluene or xylene. They also observed that cytochrome P₄₅₀ activity increased significantly in larvae exposed to test chemicals, but this did not happen in the presence of 3',4'-dimethoxy avone, a known aryl hydrocarbon receptor (AHR).

PAHs easily get absorbed in the gastrointestinal tract of mammals and are distributed in different body parts due to their high lipid solubility (Cerniglia 1984). Further, they get oxidized in presence of the cytochrome P₄₅₀-mediated mixed

function oxidase system, resulting into formation of epoxides or phenols. Further degradation of these compounds resulted into formation of sulphates, glucuronides or glutathione conjugates. Moreover, epoxides may also get metabolized into dihydrodiols leading to the formation of either diol-epoxides or soluble detoxification products after their oxidation (Stegeman et al., 2001). Naphthalene is a common micro-pollutant in potable water. It covalently binds to molecules in kidney, liver and lung tissues (LeFevre et al., 2012; Liu et al., 2010; Wang et al., 2015; Wincent et al., 2015). It is also an inhibitor of mitochondrial respiration. Acute naphthalene poisoning in humans can lead to nephrotoxicity and haemolytic anaemia. In addition, it also causes dermal and ophthalmological changes on prolonged exposure (Falahatpisheh et al., 2001). Phenanthrene is a mild allergen and a photosensitizer of human skin. It is also mutagenic to bacterial systems under specific conditions (Mastrangela et al., 1997). It induces sister chromatic exchange and inhibits junctional intercellular communication (Weis et al., 1998). Most of the hydrocarbons also affect aquatic organisms adversely on metabolic activities (Akhbarizadeh et al., 2016; Arias et al., 2009; Beg et al., 2015; Booij et al., 2016; Houde et al., 2011; Karacık et al., 2009; Li et al., 2015b; Peng et al., 2015; Perhar and Arhonditsis, 2014; Perrichon et al., 2015; Van Geest et al., 2011; Xia et al., 2015). Thus, the degradation of various petrochemical wastes is imperative for the conservation of human as well as environmental health.

2.3.1 Properties and toxic effects of some petrochemical compounds

There is a constant increase in the concentration of various petrochemical compounds and their by-products in water, air and soil (Álvarez et al., 2016; Chakraborty et al., 2015; Freije, 2015; Haghollahi et al., 2016; Hu et al., 2013; Park et al., 2002; Zhong and Zhu, 2013; Zolfaghari et al., 2016). Freije (2015) has reported that pollution of soil-water interface due to the release of these hydrocarbons into the environment is a major public health apprehension. It has also been documented that about 90% of these hydrocarbons released into the water are in the form of potentially perilous Benzene-Toluene-Ethylene-Xylene (BTEX) compounds (Janbandhu and Fulekar 2011; Souza et al., 2014). These pollutants in surface and ground water generally originate from leakage of petroleum storage tanks, spillover at production wells, refineries, pipelines, and storage and distribution terminals (Bonvicini 2014; Boopathy 2004; Hazrati et al., 2015; Karadag et al., 2016; Lan and Minh 2013; Louis et al., 2016; Rattanajongjitrakorn and Prueksasit 2014).

Mining and petrochemical industries are instrumental in the economic development of a significant number of countries worldwide and the products of these industries are considered to be the major boons to the modern society (Li 2016; Liew et al., 2014; Raufflet et al., 2014). However, the wastes generated from these industries are toxic, carcinogenic and recalcitrant, which pose serious threats to the human health and the environment (Chen et al., 2015a; Suarez et al., 2007; Yang et al., 2015). Thus, wastes from these industries have been classified as ‘hazardous’ which do not have any further use (Rovira et al., 2014). Table 1 lists various physical

and chemical properties of different petrochemical compounds, while figures 2.2 and 2.3 give chemical structures of these petrochemical compounds.

Table 2.1: Physical and chemical properties of different petrochemical compounds

| Name | Molecular formula | Molecular weight (g/mol) | Density (kg/l) | T _m (°C) | T _b (°C) | Vapour pressure (kPa) | Aqueous solubility (mg/l) | Henry's Law constant (Pa m ³ mol ⁻¹) | Log K _{ow} (-) |
|--------------------|---|--------------------------|----------------|---------------------|---------------------|-----------------------|---------------------------|---|-------------------------|
| Benzene | C ₆ H ₆ | 78.1 | 0.878 | 5.5 | 80.01 | 10.13 | 1,780 | 547 | 2.13 |
| Toluene | C ₇ H ₈ | 92.1 | 0.867 | -95 | 110.8 | 2.93 | 515 | 669 | 2.65 |
| Ethylbenzene | C ₈ H ₁₀ | 106.2 | 0.867 | -95 | 136.2 | 0.93 | 152 | 588 | 3.20 |
| ortho-Xylene | C ₈ H ₁₀ | 106.2 | 0.880 | -25 | 144.4 | 0.67 | 175 | 496 | 2.95 |
| meta-Xylene | C ₈ H ₁₀ | 106.2 | 0.864 | -48 | 139.0 | 0.80 | 200 | 699 | 3.20 |
| para-Xylene | C ₈ H ₁₀ | 106.2 | 0.860 | 13 | 138.4 | 0.87 | 198 | 709 | 3.18 |
| Phenol | C ₆ H ₆ O | 94.11 | 1.07 | 40.5 | 181.7 | 0.053 | 83000 | 0.04 | 1.46 |
| Aniline | C ₆ H ₅ NH ₂ | 93.13 | 1.0217 | -6.3 | 184.13 | 0.079 | 36000 | 0.1925 | 1.9 |
| Naphthalene | C ₁₀ H ₈ | 128.17 | 1.0253 | 78.2 | 217.97 | 0.00864 | 31.7 | 46.6 | 3.29 |
| Styrene | C ₈ H ₈ | 104.15 | 0.909 | -30 | 145 | 0.67 | 290 | 278.64 | 2.95 |
| Phenanthrene | C ₁₄ H ₁₀ | 178.23 | 1.18 | 101 | 340 | 0.0000161 | 1.6 | 2.33 | 4.55 |
| Anthracene | C ₁₄ H ₁₀ | 178.2 | 1.28 | 215.76 | 339.9 | 0.88×10 ⁻⁴ | 0.0446 | 3.95 | 4.45 |
| Benzo[a]anthracene | C ₁₈ H ₁₂ | 228.29 | 1.19 | 158 | 438 | 0.66×10 ⁻⁹ | 9.4*10 ⁻³ | 0.339 | 5.79 |
| Pyrene | C ₁₆ H ₁₀ | 202.25 | 1.27 | 145 | 404 | 0.59×10 ⁻⁶ | 0.129-0.165 | 1.205 | 4.88 |

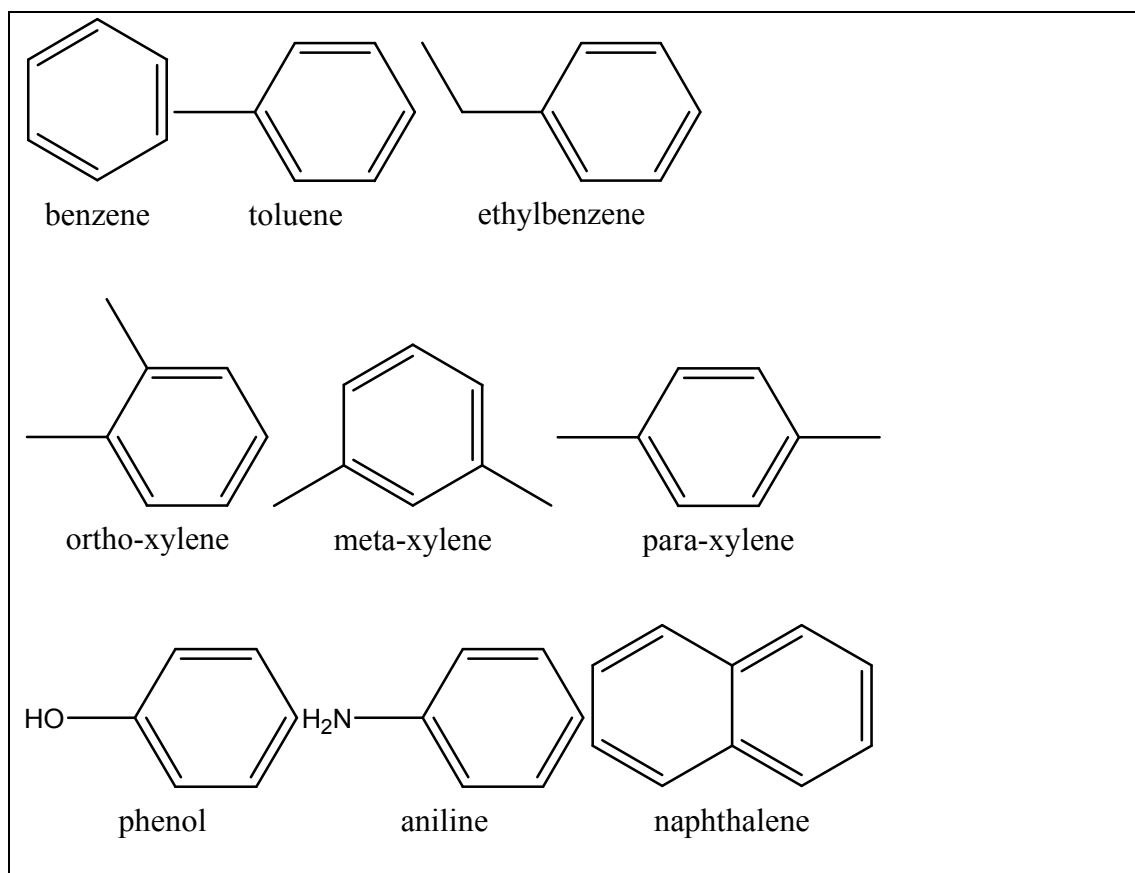


Fig. 2.2: Chemical structures of different single ringed petrochemical compounds.

2.4 Treatment technologies for degradation of petrochemical waste

The methods available and used for degradation of BTEX and other petrochemical waste products can be classified into various physical, chemical, and biological categories, depending upon the type and composition of the waste. Physical methods such as gravity separation, adsorption, membrane separation, reverse osmosis (RO), nano-filtration (NF), ultra-filtration (UF), and micro-filtration (MF) are increasingly being applied for treating oily wastewater. However, these methods have various limitations like production of large volumes of sludge, high cost of equipments, high

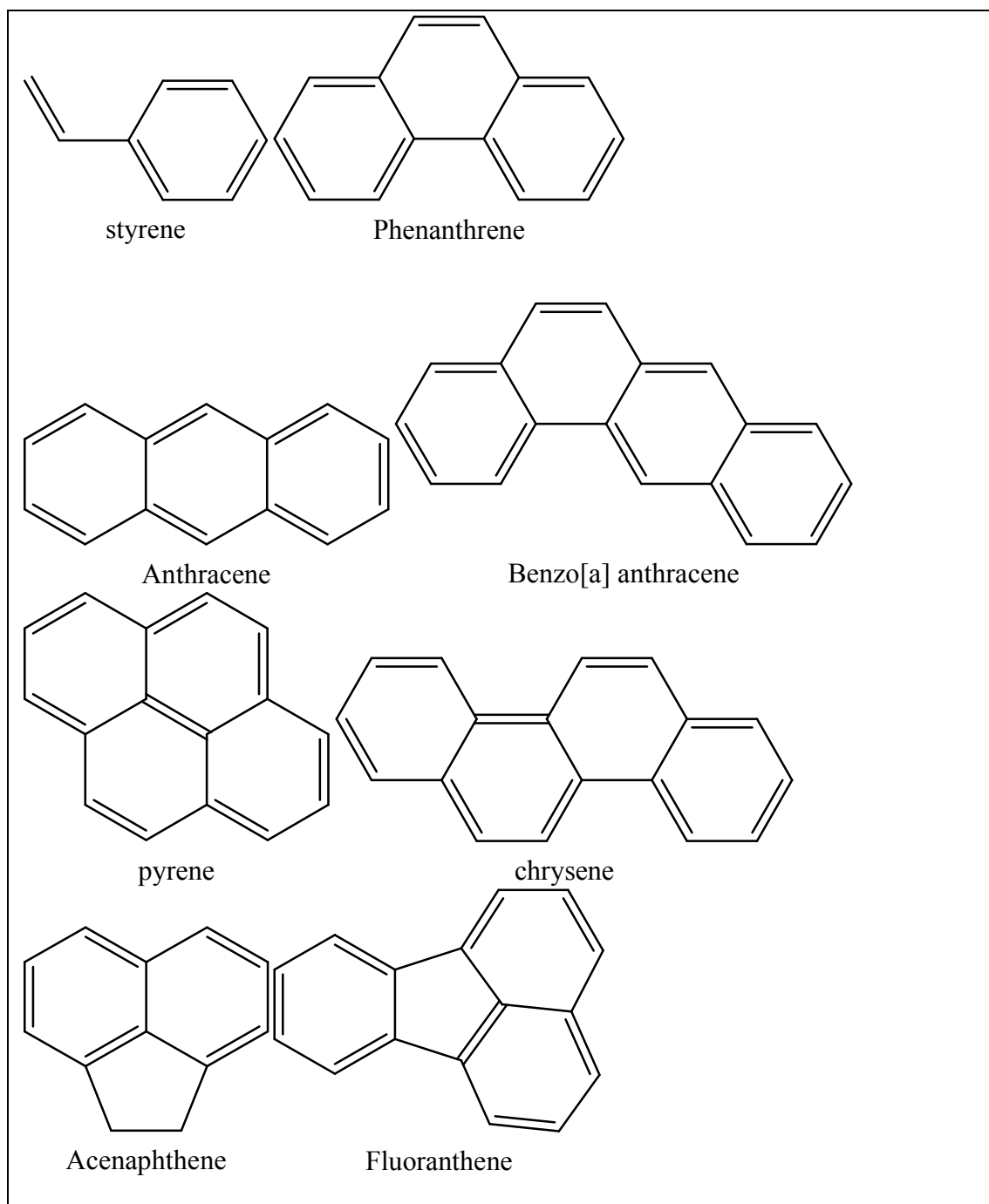


Fig. 2.3: Chemical structures of different multi-ringed petrochemical compounds.

operating costs etc. (Diya'uddeen et al., 2011; Shariati et al., 2011; Santo et al., 2012; Zhao et al., 2013). Chemical methods like precipitation, electrochemical processes, Fenton process, are applied for petrochemical waste treatment which produce low quantity of sludge but consume large quantities of chemicals, require skilled manpower and have high operation and maintenance costs (Akizuki et al., 2014; Anirudhan and Ramachandran 2014; El-Naas et al., 2014b; Hu et al., 2015; Srichandan et al., 2015; Sharma and Rangaiah 2014; Zhou et al., 2014). Biological methods used to treat petrochemical wastes include activated sludge, trickling filters, sequencing batch reactors, chemo-state reactors, biological aerated filters, bioremediation, bio- augmentation, etc. (Das and Kumar 2016; Diya'uddeen et al., 2011; Joseph and Joseph 2009; Riser-Roberts 1998; Srichandan et al., 2015; Wang et al., 2016c). Table 2.2 presents different technologies available under each of these three heads. Choice of the best technology is based on waste chemistry, cost-effectiveness, space availability, reuse and discharge plans, durable operation and by-products.

In physical methods, adsorption (El-Naas et al., 2014a) and coagulation (El-Naas et al., 2014b) are the most commonly used processes. Dissolved organics can easily be adsorbed on activated carbon, organoclay, co-polymers, zeolites and other resins. Organic compounds and some heavy metals present in petrochemical waste get adsorbed on the porous surfaces of activated carbon. Activated carbon can be used to remove soluble BTEX compounds, while organoclay can be used to remove insoluble free hydrocarbons. Organo clay is produced by the combination of sodium montmorillonite clay and a cationic ammonium or quaternary amine salt. When

organoclay and activated carbon are used in conjunction, concentration of hydrocarbons in water falls well below acceptable water quality standards. Copolymeric beads are produced by the suspension polymerization of methylmethacrylate and divinylbenzene. These copolymers can decrease oil content of petrochemical waste by almost 85%. Zeolites pellets in the form of a fixed bed can be used to adsorb dissolved organic compounds from petrochemical waste. A resin-filled column can also be used for the same purpose. In both these methods, adsorbents can be regenerated by acid backwash. But, chemical wastes from the regeneration process often present new challenges. Evaporation, dissolved air precipitation, electrodialysis, freezing and thawing are other commonly used physical treatment methods. Some advanced techniques involve the application of membrane technology (Li et al., 2006a; Rahman and Al-Malack, 2006).

The most common chemical process used for treatment of effluents is oxidation (Abdelwahab et al., 2009). However, there are various problems associated with it, such as, requirement of excessive quantity of chemicals (Guo and Al-Dahhan, 2005), low reaction rate (Huang and Shu, 1995) and production of sludge which requires further treatment. Recently, advanced oxidative processes (AOPs) such as photocatalytic degradation, microwaves assisted catalytic wet air oxidation (Sun et al., 2008), Fenton process, etc. have gained popularity. Advanced oxidative processes involve the generation of hydroxyl radical. The hydroxyl radical has a higher oxidation potential (estimated to be +2.8 V) than most other oxidants (Al-Rasheed, 2005), thus, it can degrade a very wide range of organic pollutants. Heterogeneous photocatalytic degradation has been found to be a highly effective treatment

technology (Li Puma and Yue, 2003) because of its potential to completely mineralize organic pollutants. Also, the catalyst is non-toxic, readily available and requires skilled manpower.

Besides physical and chemical methods, various biological methods such as activated sludge, trickling filters, sequencing batch reactors, chemo-state reactors, biological aerated filters, bioremediation, bioaugmentation, etc. are also used to treat petrochemical waste. Biological methods employ the diverse metabolic and enzymatic capabilities of microbes for detoxification or mineralization of pollutants. These microbes utilize the carbon contained in the petrochemical waste for their metabolism, and in the process, degrade the pollutants to less harmful products. Thus, the harmful pollutants undergo biotransformation. Biological methods have low operating costs and since they involve direct degradation of pollutants, there is no danger of generation of more toxic intermediates. Biodegradation of petrochemical waste by indigenous microbial population is one of the most eco-friendly, energy-efficient and cost-effective processes for the remediation of petrochemical pollutants. The most attractive advantage of bioremediation is the generation of basic non-harmful elements like carbon, nitrogen and hydrogen as end-products which easily get assimilated into the environment (El-Naas et al., 2014b). Also, bioremediation efficiency of indigenous microorganisms can be significantly enhanced by optimizing certain factors like adsorption, mass transfer and bioavailability (Sudip et al., 2002).

In recent times, advance oxidation processes (AOPs) such as photocatalytic degradation, ultrasonication (Levchuk et al., 2014; Petronella et al., 2016; Szreniawa-

Sztajnert et al., 2013), etc. are evolving as promising techniques for efficient sequestration of chemically stable and less biodegradable organic pollutants (Aljuboury et al., 2015; Bokare and Choi, 2014; Hasan et al., 2012; Oller et al., 2011; Shahidi et al., 2015). AOPs involve production of hydroxyl radical which attacks organic compounds and converts them into less toxic products like CO₂, H₂O and other inorganic salts (Cooper et al., 1993; Martínez-Huitle et al., 1993; Neyens and Baeyens, 2003; Oturan, 2014; Tedder and Pohland, 1993). Photocatalytic degradation of these organic pollutants has drawn much research attention (Karunakaran, 2005; Karunakaran and Senthilvelan, 2003; Li et al., 2013; Li et al., 2012; Lin et al., 2012; Manzetti et al., 2014; Qi et al., 2016; Romão and Mul 2016). In most photocatalytic degradation processes, titanium dioxide (TiO₂) powder, with its strong oxidizing tendency, acts as a photocatalyst (Barreca et al., 2015; De León et al., 2015; Lee et al., 2014; Su et al., 2016). Further, low cost and non-toxicity adds to its value and usage in broad manufacturing industries. However, its applicability and efficiency for practical use is still moderate (Han et al., 2007; Haque 2007). Photocatalytic activity of the catalysts gets escalated many times in the presence of UV radiation (Mahmoodi et al., 2011). However, the process becomes economical if sunlight is used instead of ultraviolet light for excitation of photocatalyst (Carbajo et al., 2016; Fenoll et al., 2012; Foletto et al., 2013; Liu et al., 2015; Vinoth et al., 2016; Wang et al., 2016a). Various other light sources, such as light emitting diodes (LEDs), have also been used as light source for the excitation of heterogeneous catalyst (Dai et al., 2014; Ge et al., 2015; Xiong and Hu 2016).

Table 2.2: Treatment technologies available for degradation of petrochemical waste

| Physical Methods | Chemical Methods | Biological Methods |
|---|-------------------------|---|
| Adsorption of dissolved organics on activated carbon, organoclay, co-polymers, zeolites and other resins, etc. | Precipitation | Activated Sludge |
| Coagulation | Oxidation | Trickling Filters |
| Evaporation | Electrochemical Process | Sequencing Batch Reactors |
| Dissolved air precipitation | Photo-catalytic Process | Chemostate Reactors |
| Freezing and Thawing | Fenton Process | Biological Aerated Filters |
| Electrodialysis | Ozonation | Bioremediation |
| Membrane Treatment methods like micro-filtration, ultra-filtration, nano-filtration, reverse osmosis, bentonite clay membranes, zeolite membranes, etc. | Demulsification | Phytoremediation Rhizosphere remediation |

2.5 Bioremediation of petrochemical waste

2.5.1 Mode of bioremediation of petrochemical waste

In 1928, Gray and Thronton discovered the BTEX degrading ability of microorganisms naturally occurring in soil. During bioremediation indigenous

microorganisms efficiently degrade hydrocarbon contaminants present in petrochemical waste into carbon dioxide, water, inorganic compounds and cell protein. Microorganisms are able to do so because they are acclimatized to such environmental conditions. The vulnerability of hydrocarbons to microbial degradation can generally be categorized in the following sequence; linear alkanes, branched alkanes, cyclic alkanes and small aromatics (Atlas, 1992; Lal and Khanna, 1996; Perry, 1984; Ulrici, 2000). Aromatic compounds have limited chemical reactivity and are normally attacked by fungi and bacteria with the help of enzyme oxygenases. In this process, compounds such as catechol and protocatechuate are formed as intermediates (Fuchs et al., 2011).

Bacteria are considered to be the most active and primary agents in petrochemical degradation (Atlas, 1985; Floodgate, 1984). Fungus genera are *Amorphoteca*, *Neosartorya*, *Talaromyces*, *Graphium* and that of yeast genera are *Candida*, *Yarrowia* and *Pichia* (Chaillan et al., 2004). In some cases, different microbes are used together for the degradation of petrochemical wastes. The microbial consortium of *Arthrobacter*, *Burkholderia*, *Mycobacterium*, *Pseudomonas*, *Sphingomonas*, and *Rhodococcus* has been more effective for alkyl aromatic degradation in marine sediments as compared to individual strains (Jones et al., 2007). This is ascribed to broad enzymatic capability of the consortium, which enhances the degradation rate. Various microbes used in the degradation of petrochemical waste are discussed below in detail.

2.5.1.1 Bacteria

Bacteria are the most active microbial group for the degradation of BTEX and other petrochemical pollutants. Many bacterial species have the ability to metabolize organic pollutants, but there is no single species which has the enzymatic capability to degrade all the petrochemical wastes. It is the mixed bacterial communities which have such biodegradation potential. For example, the aerobic gram-negative *Pseudomonas* shows no fermentative activities, but *Pseudomonas putida* and *Pseudomonas fluorescens* together have the highest biodegradative capacity. Aromatic hydrocarbons are inert towards simple oxidation-reduction and require elaborate degradation strategies. *Pseudomonas putida* is the most commonly used bacterium for the degradation of aromatic hydrocarbons (Alagappan et al., 2004; Otenio et al., 2005; Shim et al., 2002). It is non-pathogenic and demonstrates diverse metabolism as compared to other bacterial species. This gram negative bacterium has the ability to metabolize BTEX compounds and other aromatic hydrocarbons as the carbon and energy source (Gibson et al., 1990; Mazzeo et al., 2010). It has also been shown to function as soil inoculants to remediate naphthalene-contaminated soils (Gomes et al., 2005). It is also capable of converting styrene oil into the biodegradable polyhydroxyalkanoates (PHAs) (Ward et al., 2006).

Benzo[a] pyrene, a polycyclic aromatic hydrocarbon (PAH), is one of the most carcinogenic and toxic petrochemical pollutants. Some studies have shown that bacteria can degrade PAH when grown on an alternate carbon source in liquid culture (Ye et al., 1996). Recent studies carried out for the decontamination of PAH-

contaminated soil-water systems used a mixed culture of bacteria from genera *Acenitobacter* and *Klebsiella*. Using these bacteria, a significant reduction of up to 98% of the total PAH content took place within six months. The concentration of three and four-ringed PAHs decreased to just 0.5% of the original amount, while the concentration of the highly carcinogenic, five to seven-ringed PAHs decreased to 3% of the original content (Daane et al., 2001). The bacterium *Bacillus hexabovorum* grows aerobically in a medium containing toluene-xylene mixture and helps in the degradation of BTEX compounds. A comprehensive list of bacteria degrading petrochemical wastes is presented as table 2.3.

Table 2.3: Comprehensive list of bacteria employed to degrade different petrochemical waste products

| Sr. No. | Petrochemical compounds | Bacteria | Reference |
|---------|---------------------------------|-----------------------------------|------------------------|
| 1. | BTEX | <i>Rhodococcus rhodochorus</i> | (Deeb et al., 1999) |
| 2. | Benzene, toluene and phenol | <i>Rhodococcus rhodochorus</i> | (Yeom et al., 2001) |
| 3. | BTEX, Phenol, Isopropyl benzene | <i>Rhodococcus</i> sp. <i>DK7</i> | (Kim et al., 2002) |
| 4. | BTEX | <i>Pseudomonas putida</i> | (Attaway et al., 2002) |
| 5. | BTEX(o)-X | <i>Pseudomonas putida</i> | (Shim et al., 2002) |
| 6. | BTEX(o)-X | <i>Pseudomonas fluroescence</i> | (Shim et al., 2002) |
| 7. | Benzene | <i>Pseudomonas aeruginosa</i> | (Kim et al., 2003) |

| | | | |
|-----|-------------------------|---|-------------------------|
| 8. | BT, Phenol | <i>Pseudomonas putida</i> F1ATCC700007 | (Abuhamd et al., 2004) |
| 9. | BT | <i>Pseudomonas putida</i> F1 | (Alagappn et al., 2004) |
| 10. | BT | <i>Burcholderia (Ralstonia)</i> <i>pickettii</i> PKO1 | (Alagappn et al., 2004) |
| 11. | BT and m-xylene | <i>Rhodococcus pyridinovorans</i> PYJ-1 | (Jung et al., 2004) |
| 12. | BTX | <i>Pseudomonas putida</i> CCMI 852 | (Otenio et al., 2005) |
| 13. | BTEX | <i>Achromobacter xylooxidans</i> | (Nielsen et al., 2006) |
| 14. | BTX | <i>Pseudomonas</i> spp. | (Jean et al., 2008) |
| 15. | BTE, O-,m-,p- xylene | <i>Pseudomonas spadix</i> BD-a59 | (Kim et al., 2008) |
| 16. | BTX | <i>Alcaligenes (Achromobacter)</i> <i>xylooxidans</i> | (Shahna et al., 2010) |
| 17. | BTEX | <i>Pseudomonas</i> sp. (YATO411) <i>Mycobacterium</i> sp. (CHXY119) | (Lin et al., 2012) |
| 18. | BTEX | <i>Pseudomonas putidia</i> YNS1 | (Chang et al., 2014) |
| 19. | BTEX | <i>Bacillus sphaericus</i> (MTCC 8103) | (Mathur et al., 2013b) |
| 20. | BTEX(o)-X | <i>Mycobacterium cosmeticum</i> byf-4 | (Zhang et al., 2013) |
| 21. | BTEX | Free and mixedbacterial strain, Bb5 | (Singh et al., 2010) |
| 22. | BTEX | Free <i>Janibacter</i> sp. SB2 | (Jin et al., 2013) |

| | | | |
|-----|------------------|--|-------------------------------|
| 23. | BTEX | <i>Pseudomonas</i> sp., <i>Yarrowia</i> sp., <i>Acinetobacter</i> sp., <i>Corynebacterium</i> sp., <i>Sphingomonas</i> spp. | (Jo et al., 2008) |
| 24. | BTEX | Bacterial consortium; Strain FMB08; <i>P. putida</i> F1; and <i>Escherichia coli</i> strain DH5a | (Morlett et al., 2010) |
| 25. | BTEX | Immobilized <i>Mycobacterium</i> sp. CHXY119; <i>Pseudomonas</i> sp. YATO411 | (Xin et al., 2013) |
| 26. | BTEX | Free <i>Mycobacterium</i> <i>cosmeticum</i> byf-4 | (Zhang et al., 2013) |
| 27. | BTEX | Free and immobilized <i>P.</i> <i>Putida</i> F1 | (Robledo et al., 2011) |
| 28. | BTEX | Immobilized <i>Mycobacteriumn</i> sp. (CHXY119) and <i>Pseudomonas</i> sp. (YATO411) | (Lin et al., 2012) |
| 29. | BTEX | Free <i>Bacillus sphaericus</i> | (Mathur et al., 2013b) |
| 30. | BTEX | Free Gram-positive, Gram- negative <i>Streptomyces</i> | (Zilli et al., 2005) |
| 31. | BTEX | Free filamentous bacteria (A- 1,ATCC No.55581) | (Bielefedt et al., 1998) |
| 32. | BTEX | <i>Pseudomonas putida</i> | (Muftah et al., 2014) |
| 33. | Benzene Toluene | <i>Pseudomonas putida</i> | (Mathur et al., 2010) |
| 34. | BTE and m-xylene | <i>Pseudomonas aeruginosa</i> | (Lin et al., 2007) |
| 35. | BTEX | <i>Pseudomonas spadix</i> BD a59 | (Seung et al., 2011) |
| 36. | BTEX | <i>Dechloromonas strain</i> RCB | (Chakraborty et al., 2005) |

| | | | |
|-----|------------------------------|---|-------------------------------|
| 37. | Toluene | <i>Pseudomonas pickettii</i> PKO1. J Bacteriol | (Olsen et al., 1994) |
| 38. | Diesel glycerol | <i>Pseudomonas aeruginosa</i> J4 | (Wei et al., 2005) |
| 39. | Toluene | <i>Actinobacteria</i> and <i>Proteobacteria</i> | (Munoz et al., 2013) |
| 40. | BTEX | <i>Pseudomonas piketiii</i> and other bacterial strain (CFS215,PKO1) (Hypoxic condition) | (Olsen et al., 1991) |
| 41. | Phenol–sodium salicylate | <i>Pseudomonas putida</i> | (Juang et al., 2006) |
| 42. | B-T, B-P, T-P (mixed) | <i>Pseudomonas putida</i> F1 | (Abuhamed et al., 2004) |
| 43. | Diesel, fuels, heavy oils | <i>Pseudomonas cepacia</i> CCT6659 | (Silva et al., 2014) |
| 44. | Aniline | <i>Candida tropicalis</i> AN1 | (Dianzhan et al., 2010) |
| 45. | Aniline | <i>Pseudomonas putida</i> UCC22 (pTDN1) | (Fukumori et al., 1997) |
| 46. | Naphthalene | <i>Alcaligenes</i> | (Gomes et al., 2005) |
| 47. | Naphthalene | <i>Bacillus</i> | (Annweiler et al.,2000) |
| 48. | Naphthalene | <i>Alcaligenes denitrificans</i> | (Weissenfels et al., 1992) |
| 49. | Naphthalene | <i>Pseudomonas</i> | (Ahn et al., 1998) |

2.5.1.1.1 Aerobic degradation of petrochemical waste

Aerobic conditions provide O₂ as a powerful oxidant to oxidize and cleave the ring of aromatic petrochemical compounds. Oxygen not only serves as the final electron acceptor but also as a co-substrate for some key catabolic processes (Díaz et al., 2013; Fuchs et al., 2011). Bacteria show specificity in waste degradation. Some of them more readily degrade petrochemical compounds such as naphthalene and phenanthrene. On the other hand, *Pseudomonas paucimobilis* strain EPA 505 degrades petrochemical pollutants such as pyrene, fluoranthene, chrysene, benz[a]anthracene, benzo[a]pyrene and benzo[b]fluoranthene (Ye et al., 1996). Gram positive strains of *Mycobacterium* sp. are capable of degrading pyrene, benz[a]anthracene and benzo[a]pyrene (Schneider et al., 1996). BTEX compounds are mostly degraded by aerobic pathway. Figure 2.4 shows the main principle for aerobic degradation of hydrocarbons by microorganisms. Aerobic degradation of aromatic petrochemical waste mostly starts with the attack of dioxygenase enzymes on aromatic rings yielding cis-dihydrodiols. This is followed by dehydrogenation of cis-dihydrodiols to 1,2-dihydroxy compounds by a dehydrogenase, and finally followed by metabolism of 1,2-dihydroxy compounds (Goyal et al., 1997).

Benzene gets directly degraded to catechol. Toluene can be degraded by many different biodegradative pathways, some involving 3-methylcatechol as an intermediate. Similarly, many different biodegradative pathways exist for the degradation of ethylbenzene, involving 3-ethylcatechol as an intermediate in some

cases. Degradation of xylene is a complex process because all xylenes get metabolized to mono-methylated catechols,. The aromatic ring of all these substituted catechols is later cleaved under the action of dioxygenase enzymes. Aerobic degradation of alkylated phenols is accomplished by either of the two pathways: (1) hydroxylation to catechols, followed by extradiol ring fission, and (2) oxidation of the methyl group to carboxyl followed by hydroxylation to hydroquinones and fission by the gentisate pathway (Bayly et al., 1988). Chemo-organotrophic species of bacteria degrade the organo pollutants present in the oxic zone of contaminated sites by using a large number of natural and xenobiotic compounds as carbon sources and electron donors for energy generation (Evans et al., 2003).

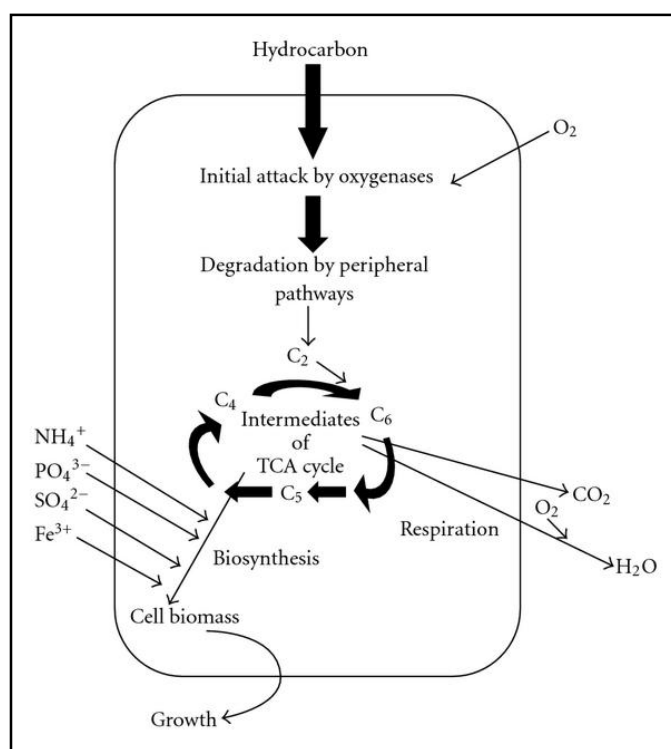


Fig. 2.4: Aerobic degradation of hydrocarbons by microorganisms (Adopted from Das et al., 2010).

2.5.1.1.2 Anaerobic degradation of petrochemical waste

Petrochemical compounds are often present in anoxic zones of contaminated sites and warrant the need of understanding anaerobic bioremediation. Anaerobic degradation of petrochemical waste has been studied by a number of investigators (Balachandran et al., 2012; Chang et al., 2003; Feng et al., 2014; Grbic-Galic et al., 1991; Heider et al., 1999; Meckenstock and Mouttaki, 2011; Meckenstock et al., 2004; Schink et al., 1992; Sharma and Philip, 2014; Stasik et al., 2015; Venkateswar Reddy et al., 2015). Varieties of microorganisms such as bacteria, fungi and algae have the ability to degrade BTEX and other petrochemical pollutants in anaerobic conditions. Anaerobic degradation employs certain intermediates which contain substituent having electron withdrawing effect to facilitate electron transfer to the ring. The most common intermediate is benzoyl-CoA in which carboxy-thioester acts as the electron withdrawing group (Fuchs et al., 2011).

Anaerobic biodegradation of BTEX compounds is important because BTEX compounds are mostly found in oxygen-limited conditions such as in the sediments of all natural water bodies and sometimes, even in soil. In fact, benzoyl-CoA is the common central intermediate in anaerobic biodegradation of benzene, toluene and ethylbenzene (Heider et al., 1997). *Dechloromonas* spp. completely mineralizes benzene with the help of benzoyl-CoA under anaerobic conditions (Chakraborty and Coates, 2005). The aromatic ring of benzoyl-CoA gets reduced and eventually

transformed to enzyme acetyl-CoA. Some microorganisms have the capability to anaerobically metabolize xylene. These microbes are strains of denitrifying bacteria which are capable of using m-xylene as a form of their growth substrate (Harwood et al., 1997). *Dechloromonas* RCB has the ability to degrade all the three isomers of xylene under anaerobic conditions (Chakraborty et al., 2005). But this pathway of anaerobic degradation of xylene is not well-known. Biodegradation of phenol in anaerobic conditions generally proceeds with carboxylation of phenol, followed by its dehydroxylation and finally, fission of the ring after partial reduction (Brackmann and Fuchs, 1993). Figure 2.5 represents the mechanism for the anaerobic degradation of aromatic hydrocarbons (Fuchs et al., 2011).

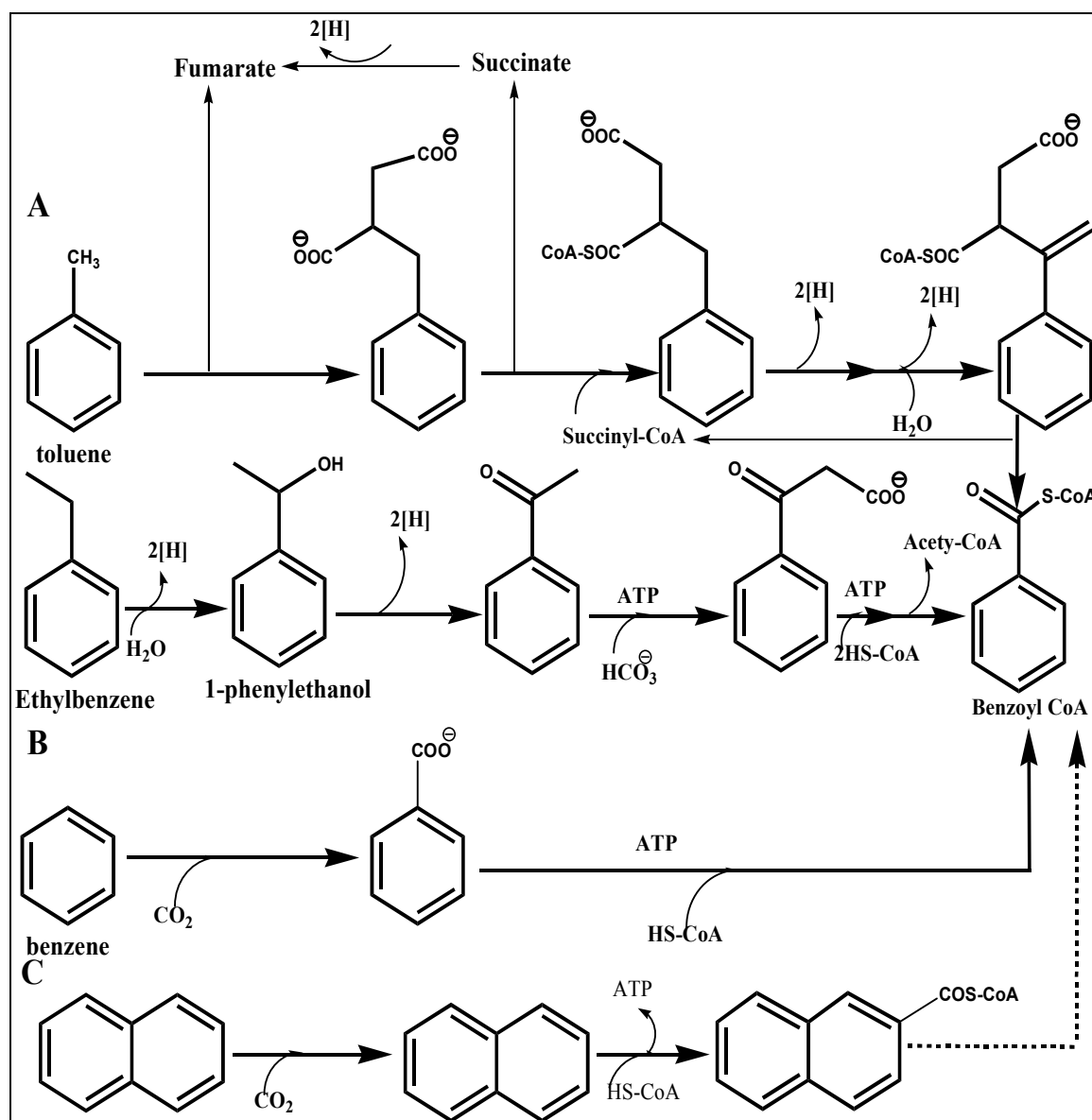


Fig. 2.5: Anaerobic degradation of aromatic hydrocarbons: (A) Fumarate addition to toluene, and subsequent β -oxidation of benzylsuccinate to benzoyl-CoA. (B) Hydroxylation of ethylbenzene to 1-phenylethanol, and the ATP-dependent carboxylation of acetophenone. (C) The proposed carboxylation of benzene and naphthalene as the initial step of anaerobic degradation.

2.5.1.2 Fungi

It has been reported in the earlier studies that fungi can be used for biodegradation of petrochemical waste. Fungi have the enzymatic ability to degrade a vast variety of structurally and functionally diverse petrochemical wastes *via* pathways which are similar to those of mammalian enzyme systems (Národný et al., 2002). Fungi play a significant role in biodegradation of recalcitrant environmental pollutants because they are capable of withstanding harsher environmental conditions than other microorganisms. Petrochemical pollutants have chemical structures analogous to naturally-occurring compounds which act as carbon sources for fungi. Thus, fungi have the ability to use petrochemical pollutants as potential carbon sources (Norton, 2012). Some wood rotting fungi such as oyster mushrooms, *Pleurotus ostreatus*, has been reported to degrade almost 80-95% of all PAHs present in soil in a period of 80 days (Steffen and Shubert, 2007). Several fungal species oxidize PAHs *via* a cytochrome P₄₅₀ mono-oxygenase enzyme to form an arene oxide and other atoms into a water molecule. Several groups of non-ligninolytic fungi have the ability to oxidize petro-carbons to trans-dihydrodiols, phenols, tetralones, quinones, dihydrodiol epoxides and different conjugates of hydroxylated intermediates. However, only a few fungi have the ability to degrade petro-carbons to CO₂ (Prenafeta-Bold et al., 2001). Fungus *P. chrysosporium* degrades all BTEX components simultaneously and this degradation is supported under non-ligninolytic culture conditions when no LIPs and MNPs are produced (Yadav and Reddy, 1993). Table 2.4 presents a comprehensive list of fungi employed to degrade different petrochemical waste products.

Table 2.4: Comprehensive list of fungi employed to degrade petrochemical wastes

| Sr. No. | Petrochemical compounds | Fungi | Reference |
|---------|-------------------------|--|--------------------------------|
| 1. | BTEX | Free Fungus <i>Paecilomyces variotii</i> CBS115145 | (Garcia-Pina et al., 2008) |
| 2. | BTEX | <i>Phanerochaete chrysosporium</i> | (Yadav et al., 1993) |
| 3. | TEX | <i>Cladophialophora</i> sp. T1 | (Prenafeta-Boldu et al., 2002) |
| 4. | TE, (o-,m-,p-)X | <i>Cladosporium</i> sp. <i>Cladophialophora</i> sp. | (Nikolova et al., 2005) |
| 5. | BTE, (o-,m-,p-)X | <i>Paecilomyces variotii</i> CBS115145 | (Garcia-Pina et al., 2008) |
| 6. | TEX | <i>Cladophialophora psammophila</i> | (Badali et al., 2011) |
| 7. | Phenol-resorcinol | <i>Gliomastix indicus</i> MTCC 3869 | (Kumar et al., 2013) |
| 8. | Phenol-m-cresol | <i>Alcaligenes faecalis</i> | (Bai et al., 2007) |
| 9. | Phenol-resorcinol | <i>Trichosporon cutanium</i> R57 | (Aleksieva et al., 2002) |
| 10. | Phenol-p-cresol | <i>Trametes versicolor</i> | (Alexieva et al., 2010) |
| 11. | Phenol-p-cresol | <i>Trichosporon cutaneum</i> | (Alexieva et al., 2008) |
| 12. | Phenol-p-cresol | <i>Aspergillus awamori</i> | (Yemendzhiev et al., 2008) |
| 13. | Phenol-m-cresol | <i>Candida albicans</i> PDY-07 | (Wanget al., 2008) |
| 14. | Styrene | <i>Exophiala jeanselmei</i> | (Cox et al., 1997) |
| 15. | Styrene | <i>E. jeanselmei</i> | (Jung et al., 2005) |

2.5.1.3 Genetically Modified Organisms (GMOs)

Genetically Modified Organisms (GMOs) can be used for effective and economically feasible bioremediation processes. Under laboratory conditions, genetically engineered microorganisms immensely improve the biodegradation efficiency of petrochemical waste. GMOs, such as *Pseudomonas* sp, *P. fluorescens*, *P. pseudoalcaligenes* and *Pseudomonas putida* play an important role in the degradation of petrochemical waste (Das and Chandran, 2010). Genetically modified bacteria and fungi, are reported to degrade recalcitrant, xenobiotic organic compounds from the environment. They can also be used for the monitoring of stress-strain response and toxicity assessment in biodegradation processes. However, various environmental and ecological concerns are the major obstacles for testing of GMOs in the field.

2.5.1.4 Role of enzymes in biodegradation of petrochemical wastes

Biodegradation of BTEX compounds and other aromatic petroleum hydrocarbons involves several enzymes. Some of these are oxygenase, dehydrogenase, cytochrome P₄₅₀ enzyme and soluble methane monooxygenases. These enzymes are critically involved in the catalytic transformation of petrochemical pollutants (Huang, 2004). Soluble methane monooxygenase enzyme from microbes like *Methylococcus*, *Methylosinus*, *Methylocystis*, *Methylomonas* and *Methylocella* helps in the biodegradation of C₁–C₈ alkanes, alkenes and cycloalkanes. AlkB-related alkane hydroxylase extracted from *Pseudomonas*, *Burkholderia* and *Rhodococcus mycobacterium* are used for the degradation of C₅–C₁₆ alkanes, fatty acids, alkyl

benzenes and cycloalkanes (Van et al., 2007). Eukaryotic P_{450} enzymes from *Candida maltose*, *Candida tropicalis* and *Yarrowia lipolytica* enhance the degradation of petroleum hydrocarbons such as C10–C16 alkanes and fatty acids (Lida et al., 2000). Bacterial P_{450} oxygenase system (Van et al., 2007) and certain dioxygenases (Sakai et al., 1996) are also involved in the degradation of hydrocarbons. Bacteria carrying catabolic plasmid DNA in addition to chromosomal DNA are more efficient in degrading petrochemical contaminants than the catalase-negative bacteria (Boronin and Kosheleva, 2014; Das et al., 2010).

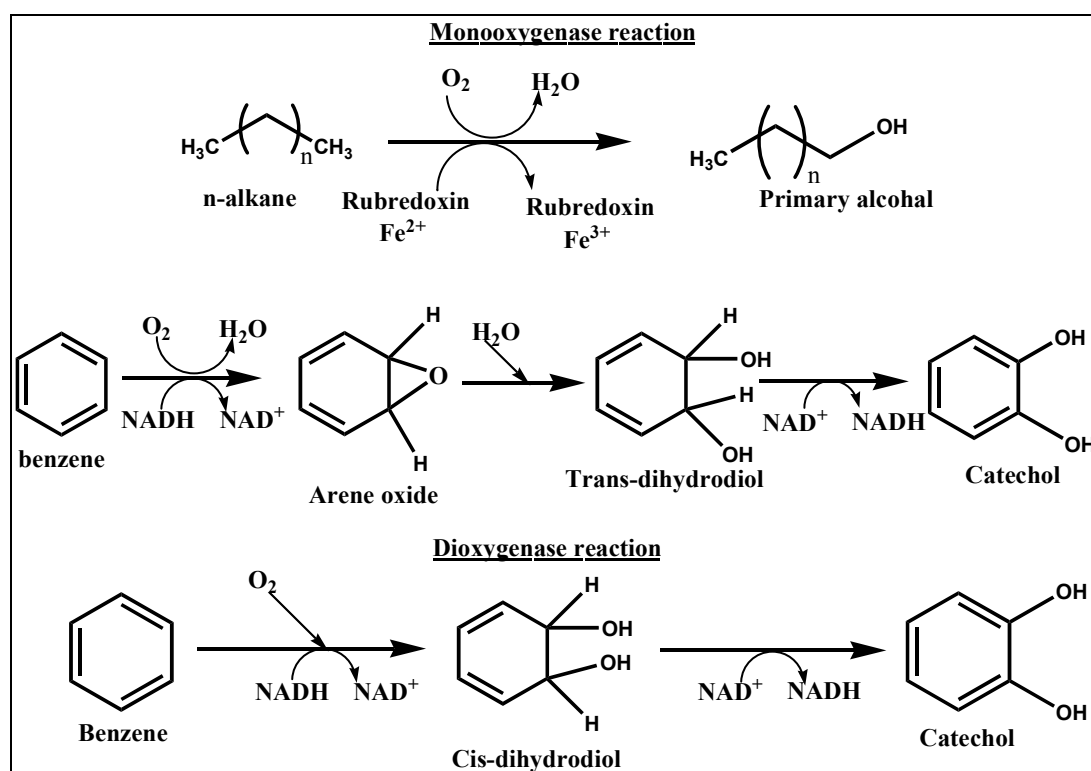


Fig. 2.6: Action of monooxygenase and dioxygenase enzymes in the biodegradation of petrochemical wastes.

The fungal ligninolytic enzymes consists of lignin peroxidase, laccase and manganese peroxides (Haritash and Kaushik, 2009). They catalyze radical formation by oxidation leading to destabilization of bonds in hydrocarbon molecules and ultimately lead to their degradation (Ball et al., 1995; Hofrichhter et al., 1999). *Pseudomonas* spp. also produces a number of extracellular enzymes. These enzymes can be immobilised on clay and soil colloids, leading to more stability in field conditions, and thus, gives better results (Chang et al., 2015; Lee et al., 2011; Singh et al., 2003). Singh et al. (2003) characterised the extracellular enzyme of *Brevibacterium* spp. intercalated into a surfactant modified montmorillonite, which could degrade fenamiphos, an organophosphorous pesticide. Clay colloids-protected extracellular enzymes have a longer functionality than their free states, but sometimes, their activity level may get reduced (McAllister and Semple, 2010). The action of monooxygenase and dioxygenase enzymes in the biodegradation of petrochemical compounds is shown figure 2.6.

2.5.1.5 Biosurfactants enhanced biodegradation of petrochemical waste

Biodegradation of petrochemical waste is limited by various factors *viz.*, their hydrophilic nature, low solubility in aqueous media and low dissolution rate. Surfactants can be used to overcome these limitations. Surfactants are either of chemical or biological origin. They help in degradation by solubilization or emulsification of hydrocarbons. Surfactants enhance the degradation of toxic petrochemical compounds by increasing their solubility in water thereby increasing their bioavailability (Alvarez and Vogel, 1995). Microbial compounds which exhibit

particularly high surface activity and emulsifying activity are classified as biosurfactants. These are structurally diverse compounds, mainly produced by the hydrocarbon-utilizing microorganisms that exhibit surface activity. They possess different chemical structures such as lipopeptides, glycolipids, neutral lipids and fatty acids. They are nontoxic, bimolecular and often very good emulsifiers. They have both hydrophobic and hydrophilic domains and are capable of lowering the interfacial tension of the growth medium (Cametora and Bollag, 2010).

Studies have shown that the degradation of petroleum hydrocarbons gets greatly enhanced by the use of biosurfactant molecules (Al- Qin et al., 2012; Bachmann et al., 2014; Bahry et al., 2013; Hassanshahian, 2014; Luna et al., 2015; Marchant and Banat, 2012; Sajna et al., 2015; Silva et al., 2014; Souza et al., 2014; Yan et al., 2012; Yan et al., 2011). Biosurfactant producing bacteria generate rhamnolipid, sophorose lipid, glycolipid, emulsan and other crude surfactants which are responsible for the degradation of petrochemical waste (Makkar and Rockne, 2003). *Pseudomonas aeruginosa* produces rhamnolipid to efficiently degrade naphthalene, fluorene, phenanthrene, pyrene and many other PAHs (Dyke et al., 1993; Mata-Sandoval et al., 2000; Ortega et al., 2015; Qi et al., 2015; Singh et al., 2009; Xia et al., 2014). Biosurfactants desorb PAHs from soil surfaces by reducing the interfacial tension of solid-liquid phases (Szulc et al., 2014). They also increase the water solubility of PAHs, especially when present above the critical micelle concentration (Yin et al., 2009). Thus, either surfactant-producing microorganisms or biosurfactants can be added to the soil to enhance the growth of added or indigenous microorganisms (Banat, 1995; Bartha, 1986). There are many advantages of replacing

chemical surfactants with biosurfactants. Biosurfactants are biodegradable, relatively simple, inexpensive to produce and non-toxic or less toxic as compared to chemical surfactants. Besides, biosurfactants are very effective even at low concentrations (Bartha, 1986; Desai, 1987; Dyke et al., 1993).

2.5.1.6 Factors affecting microbial degradation of petrochemical waste

Owing to its toxic, hydrophobic and multi-phased nature, bioremediation of petrochemical waste is a complex phenomenon. Studies on biodegradation of petrochemical waste have identified a number of physical, chemical and biological factors which affect the rate of biodegradation. These factors include temperature, pH, availability of inorganic nutrients, pollutant concentration, soil and water composition, microbial adaptation, etc. (Bosma et al., 1997; Singh and Celin, 2010; Tang et al., 2005; Venkata Mohan et al., 2006).

2.5.1.6.1 Temperature

Temperature plays a critical role in hydrocarbon degradation as it affects microbial metabolism pathways (Margesin et al., 2001). The most optimum temperature for degradation of petrochemical waste is 30-40 °C for soil environments, 15-20 °C for marine environments and 20-30 °C for aqueous environments (Bartha et al., 1984; Mueller et al., 1989). At lower temperatures, microbial growth and propagation slows down which in turn, slows the rate of petrochemical degradation. Petrochemical compounds become more soluble with an increase in temperature of the medium, and thus, more bioavailable to the microorganisms (Gibb et al., 2001).

Some thermophilic microorganisms have the ability to degrade phenanthrene, naphthalene and anthracene under thermophilic conditions. The thermophilic bacteria *Bacillus thermoleovorans* can degrade naphthalene at 60 °C. It shows a significantly different metabolites and metabolic pathway than bacteria (Annweiler et al., 2000).

2.5.1.6.2 pH

Medium pH value plays an important role in the biodegradation of petrochemical waste. It affects the degradation activity of introduced microorganisms, both in the soil and aqueous media. Various studies have shown that the mineralization of petro-hydrocarbons is favoured near neutral (pH = 7). A little change in pH can have dramatic effects on the biodegradation processes even at low concentrations of pollutants in aquatic environments. However some fungal species are more tolerant towards acidic conditions. Various acidophilic microorganisms are capable of degrading petrochemical waste in extremely acidic conditions (Stapleton et al., 1998).

2.5.1.6.3 Nutrient availability

Microbes involved in the biodegradation processes need nutrients such as nitrogen, phosphate, potassium and carbon for their cellular metabolism and growth. In contaminated areas such as soils and water, where the levels of organic carbon are high due to the nature of the pollutants, available nutrients such as nitrogen, phosphorous, potassium and, in some cases, iron deplete rapidly during microbial metabolism. Hence, it becomes the limiting factor in the microbial degradation of

petroleum hydrocarbons (Cooney et al., 1984; Hamme et al., 2003). However, excessive concentration of nutrients can also inhibit the biodegradation activity of microorganisms (Chaillan et al., 2006). The negative effects of high concentration levels of nitrogen, phosphate and potassium on aromatic hydrocarbon degradation have also been reported (Carmichael et al., 1997).

2.5.1.6.4 Oxygen availability

Availability of oxygen determines whether the system is aerobic or anaerobic. Degradation of petrochemical hydrocarbons mainly occurs under aerobic conditions. However, in anaerobic environment such as in aquifers and marine sediments, anaerobic biodegradation is reported (Coates et al., 1996). The anaerobic biodegradation was initially considered limited to halogenated aromatic compounds like halo-benzoates, chlorophenols, etc. (Boyd et al., 1984). Bioremediation of organic contaminants such as BTEX, phenols, etc. can happen under both aerobic and anaerobic conditions. In aerobic petrochemical metabolism, oxygen is an integral component for the action of mono- and dioxygenase enzymes in the initial oxidation of the aromatic ring. In the absence of oxygen molecule, alternative electron acceptors such as nitrate, ferrous and sulphate ions are necessary to oxidize aromatic compounds. Recent studies clearly indicate that petrochemical biodegradation can occur under both denitrifying and sulphate reducing, anaerobic conditions (Zhang et al., 2000). When electron acceptors like nitrate, ferrous and sulphate ions are reduced under anaerobic conditions, it results in the release of excess phosphorous and ferrous ions in the surroundings which proves toxic to the environment. During anaerobic

degradation of petrochemical hydrocarbons, release of greenhouse gases like methane, nitrogen dioxide, etc. and an increase in pH has also been observed (Bamforth et al., 2005).

2.5.1.6.5 Bioavailability

Bioavailability of organic pollutants to the microbes is an important factor which determines the rate of biodegradation (Guthrie et al., 1998; Weissenfels et al., 1992). Solubility and bioavailability of hydrocarbons depend upon their physical nature and chemical composition.

2.5.1.6.6 Adaptation of microorganisms

Microbial adaptation during petrochemical waste biodegradation has been widely studied because it significantly affects the biodegradation pattern of petrochemical waste. Microbial communities adapt to specific carbon sources and are able to effectively degrade these specific compounds. The study by Yeom et al. (1997) reported how *Alcaligenes xylosoxidans* Y234 degraded benzene, toluene and m-xylene better than non-adapted and toluene-adapted cells. The study discusses this phenomenon in terms of catechol 1,2-dioxygenase induction. Cells adapted to benzene induced more catechol 1,2-dioxygenase than those adapted to toluene which led to the rapid degradation of benzene, toluene and m-xylene (Yeom et al., 1997). Another study by Babaarslan et al., (2005) utilized a mixed culture of microorganisms obtained from petroleum production wells. The study showed that biodegradation of toluene by non-adapted microorganisms was fastest in a single component

environment; however, that of ethyl benzene, was in a multi-component environment. They also reported that toluene-adapted cells had higher removal ability in single component environment than the non-adapted and benzene-adapted microorganisms (Babaarslan et al., 2005).

2.5.1.6.7 Concentration of pollutants

Degradation rates of individual contaminants alter due to substrate interactions which can be either synergistic or antagonistic (Wang et al., 2007). Synergistic interactions improve the degradation rates of individual contaminants with the help of catabolic enzymes. The study of substrate interaction at different concentrations is also important because cells experience substrate toxicity, especially at higher concentrations. A study by Li et al. (2006) showed how an increase in benzene concentration had inhibitory effect on the rate of biodegradation (Li et al., 2006). Another study by Abu Hamed et al., (2003) showed that the growth rate of *P. putida* in batch systems was a decreasing function of substrate concentration (Abu Hamed et al., 2003). When the BTEX compounds are in high concentrations, microbial activity gets inhibited due to various complex micro- and macro-level interactions. A study by Mathur (2010) showed how much smaller rate of biodegradation was observed when the initial concentration of benzene and toluene was higher (Mathur, 2010).

2.5.1.7 Limitations of bioremediation

While bioremediation shows wonderful results in the laboratory, it suffers from various limitations under the harsh field conditions. Selection of microorganisms

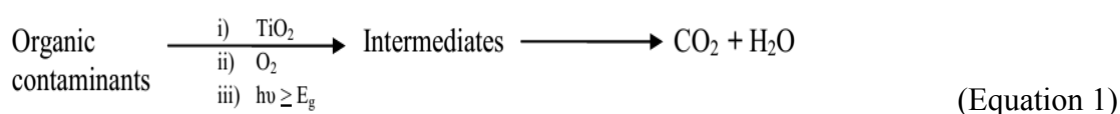
is one of the most critical factors for the efficient degradation of petrochemical waste in a particular habitat because only certain adapted microbial species would work in those specific habitats. So, it is important to isolate locally adapted strain from the particular habitat. In laboratory experiments, considerably homogeneous contaminant concentrations are used, but this is not the case in the field where the pollutants are heterogeneously distributed. Microbes get stimulated by these heterogeneously distributed contaminants to either move towards them or flee from them. Studies have revealed that pollutants act as stressors, rather than as nutrient source, if present above a certain threshold (Dominguez et al., 2006; Roca et al., 2008). Bacteria have surface sensors that make them move towards or away from the pollutants.

Another problem in using microbes is the efficiency of degradation. Generally, single microbial strains are not very effective at degrading petrochemical waste. However, microbial consortia (Isaac et al., 2013; Mao et al., 2012; Moscoso et al., 2012; Wang et al., 2010) and coupling of bacteria with fungi (Borràs et al., 2010; Schamfuß et al., 2013; Wang et al., 2012) can effectively degrade the polycyclic aromatic hydrocarbons and volatile organic compounds. Other limiting factors in the biodegradation of petrochemical waste are their hydrophilic nature, low solubility in aqueous media and low dissolution rate. Low solubility of petrochemical contaminants in aqueous media limits their bioavailability. These limitations can be overcome by the use of surfactants. Cell-to-cell communication in the environment proves to be another major limitation in the process of bioremediation.

2.5.2 Photodegradation

2.5.2.1 Advanced Oxidative Processes (AOPs)

Advanced oxidative processes (AOPs) are evolving as promising techniques towards efficient sequestration of chemically stable and less biodegradable organic pollutants (Hu and Long 2016; Ma et al., 2014; Misra 2015; Mohapatra et al., 2014; Parilti and Atkin 2010; Serpone et al., 2010). These oxidation processes are proved to be another alternative for degradation of petrochemical wastes and are regarded as environmental clean-up technologies which are both efficient and cost effective (Chen et al., 2015a; Ramteke and Gogate 2015; Saritha et al., 2007; Shahidi et al., 2015; Tisa et al., 2014; Vaferi et al., 2014; Zangeneh et al., 2014). AOPs are dependent on generation of highly reactive radical species, mainly the hydroxyl radical (OH^\bullet), by using various sources of energy. This hydroxyl radical attacks organic compounds and causes their mineralization into CO_2 , H_2O and other inorganic salts (Karci et al., 2013; Xiao et al., 2016). This mechanism is depicted in figure 2.7. The series of reactions that are involved in generation of radical species involve the absorption of radiation quanta by titania and generation of electron-hole pair. The hole breaks the H_2O into H^+ and OH^\bullet radical and electron generates oxygen radical as shown in figure 2.8. Active radical species generated are OH^\bullet , HO_2^\bullet and O_2^\bullet . These radicals have tendency to oxidize the organic moieties. The most attractive feature of AOPs is that they can degrade a wide range of organic pollutants with no selectivity (Suhadolnik et al., 2016; Xiao et al., 2016).



Thus, AOPs are the economically feasible processes, which lead to the complete mineralization and production of lesser quantities of sludge from the degradation of various organic contaminants (Akpan and Hamid 2009; Nath et al., 2016; Rajeshwar et al., 2008; Wang et al., 1999).

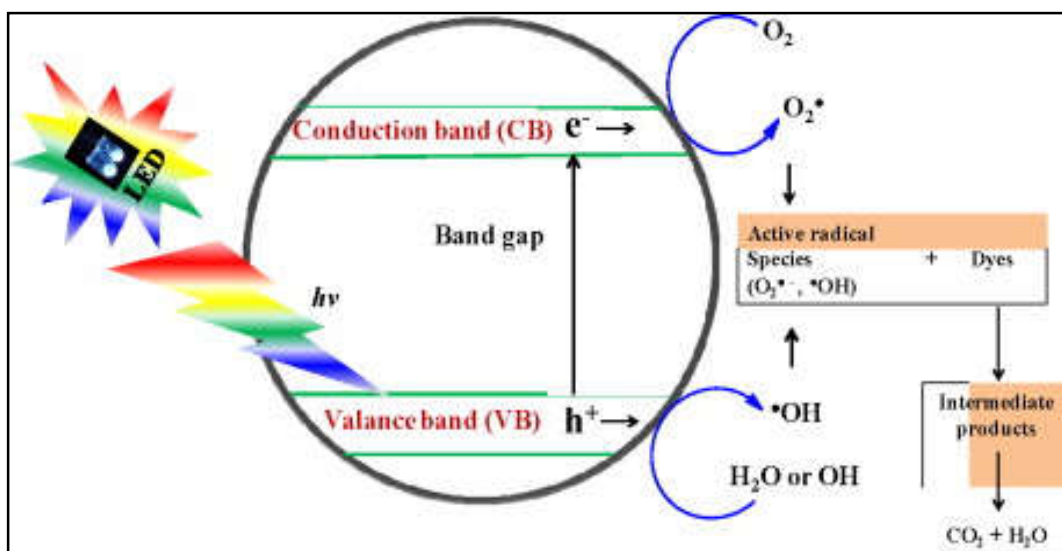


Fig. 2.7: Mechanism of photocatalytic degradation (Adapted from Jo and Tayade 2014 with permission).

Table 2.5: Various catalysts used for degradation of organic pollutants in photochemical degradation

| Catalyst | Targeted compound | Degradation efficiency | Light source | Reference |
|-------------------------------|---|------------------------|--------------|-------------------------|
| Carbon/nitrogen-doped TiO_2 | Phenol | 64 (after 30 min.) | UV | (Abdullah et al., 2016) |
| V_2O_5/TiO_2 | 1,3,5-Trichlorobenzene chlorinated benzenes | 60 58 | UV | (Wang et al., 2015) |

| | | | | |
|--|-----------------|--------------------|---|------------------------------|
| TiO ₂ nanoparticles | Gaseous benzene | - | UV | (Wang and Wu, 2015) |
| MnFe ₂ O ₄ | Gaseous benzene | - | Visible | (Shen et al., 2015) |
| TiO ₂ /SiO ₂ /Bi ₂ O ₃ | Benzene | 96 (after 7 hours) | UV-Visible | (Ren et al., 2015) |
| TiO ₂ modified by transition metals | Gaseous benzene | 58 (in 20 minutes) | vacuum ultraviolet (VUV) | (Huang et al., 2015) |
| TiO ₂ nanoparticles doped with CeO ₂ and supported on SiO ₂ | Phenol | | Visible | (Hao et al., 2015) |
| N-doped mixed TiO ₂ and ZnO | BTX | 80 (in 120 min) | Visible | (Ferrari-Lima et al., 2015) |
| Carbon-doped TiO ₂ nanoparticles wrapped with nanographene | Phenol | | Visible | (Yu et al., 2014) |
| Nano-ZnO, TiO ₂ and ZnO–TiO ₂ composite | Phenol | 70–80 | UV light irradiation and direct sun light | (Prabha and Lathasree, 2014) |
| N–H–TiO ₂ photocatalyst by annealing in NH ₃ and H ₂ | Benzene | 100 (within 3 h) | visible light irradiation | (Li et al., 2014) |
| Pd-deposited TiO ₂ fil | Gaseous toluene | 94 | UV254+185 nm | (Kim et al., 2014) |
| TiO ₂ /SiC nanocomposite fil | Toluene | 100 | UV LED | (Zou et al., 2013) |
| TiO ₂ /SiO ₂ | Benzene | 92.3 | Mercury lamp | (Liu et al., 2013) |

| | | | | |
|---|--------------------|-----------------|--------------|-------------------------------|
| Ca ₂ Nb ₂ O ₇ nanopolyhedra and TiO ₂ | Benzene | | UV | (Liang et al., 2013) |
| Perlite granules coated with in-doped TiO ₂ | Ethylbenzene | | UV | (Hinojosa-Reyes et al., 2013) |
| N-doped TiO ₂ | Benzene | | UV | (He et al., 2013) |
| Au/ZnO nanocomposites | Benzene | | UV | (Yu et al., 2012) |
| Mg-ferrite/hematite/PANI nanospheres | Benzene | 62 and 52 | Visible | (Shen et al., 2012) |
| CNT/Ce-TiO ₂ | Phenol | 94 | UV | (Shaari et al., 2012) |
| W-doped TiO ₂ | BTEX | 100 | Visible | (Sangkhun et al., 2012) |
| Degussa P25 TiO ₂ | Phenol | | UV | (Royae et al., 2012) |
| Pt-TiO ₂ /Ce-MnOx | Benzene | | | (Ren et al., 2012a) |
| Pt-loaded TiO ₂ /ZrO ₂ | | 90 | Thermo photo | (Aarthi et al., 2007) |
| BiPO ₄ catalysts | Benzene | | UV | (Long et al., 2012) |
| CdS-sensitized TiO ₂ fil | Benzene | 92.8 | UV | (Liu et al., 2012a) |
| Fe/TiO ₂ | 2,4-dichlorophenol | 97 (in 2 hours) | UV | (Liu et al., 2012b) |
| TiO ₂ -based catalyst | BTEX | | UV | (Korologos et al., 2012) |

| | | | | |
|--|--------------------|-------|-------------------------------|----------------------------|
| TiO ₂ -based catalyst | BTEX | | UV | (Korologos et al., 2012) |
| zirconium-doped TiO ₂ /SiO ₂ | Toluene and xylene | | UV | (Kim et al., 2012) |
| Ag–AgBr–TiO ₂ | Benzene | | UV-visible | (Zhang et al., 2011a) |
| SnO ₂ –TiO ₂ | Phenol | 99.8 | UV | (Zhang et al., 2011b) |
| TiO ₂ | Benzene | | UV | (Xie et al., 2011) |
| TiO ₂ | Phenol | | UV | (Lin et al., 2011) |
| Pt/TiO ₂ | Benzene | 86 | UV | (Li et al., 2011) |
| BiVO ₄ /TiO ₂ | Benzene | 92 | Visible ($\lambda > 450$ nm) | (Hu et al., 2011) |
| Micro-nano size TiO ₂ | Pyrene | 78.3 | Sun light | (Chang Chien et al., 2011) |
| TiO ₂ | Benzene | 60–70 | | (Bui et al., 2011) |
| Ti-HMS | Benzene | | UV (4 W) | (Zhuang et al., 2010) |
| TiO ₂ nanoballs | Gaseous benzene | | UV | (Aarthi et al., 2007) |
| TiO ₂ /Ce _x Zr _{1-x} O ₂ | Benzene | | | (Zhong et al., 2010) |
| nano-TiO ₂ /ZSM-5/silica gel (SNTZS) | Phenol | 90 | UV | (Zainudin et al., 2010) |

| | | | | |
|--|---|----|--------------------|---------------------------------|
| CNT in a TiO ₂ matrix | Benzene aqueous phase | | near-UV to visible | (Silva and Faria, 2010) |
| Ag ₄ V ₂ O ₇ and Ag ₃ VO ₄ phases | Benzene | 48 | | (Chen et al., 2010) |
| S, C, SnO ₂ -codoped TiO ₂ | Phenol | | Visible | (Chen et al., 2010) |
| Pd/TiO ₂ | Benzene | 60 | UV. | (Zhong et al., 2009) |
| Graphite-supported TiO ₂ | 4-nitrophenol | | UV | (Palmisano et al., 2009) |
| TiO ₂ /BaAl ₂ O ₄ :Eu ²⁺ ,Dy ³⁺ | Benzene | | UV | (Li et al., 2009) |
| Hydroxide ZnSn(OH) ₆ | Benzene | | UV (Under 254 nm) | (Fu et al., 2009) |
| CNT-doped TiO ₂ Electrodes | Phenol | 58 | Visible | (Chen et al., 2009) |
| TiO ₂ Catalyst | Phenol, Chlorobenzene and Toluene in aqueous medium | | UV | (Bougheloum and Messalhi, 2009) |
| TiO ₂ | 1,4-dichlorobenzene | | UV | (Selli et al., 2008) |
| TiO ₂ -based materials | Propene and benzene | | UV | (Bouazza et al., 2008) |
| TiO ₂ /Sr ₂ CeO ₄ | Benzene over | | UV | (Zhong et al., 2007a) |
| TiO ₂ /Sr ₂ CeO | Benzene | | | (Zhong et al., 2007b) |

| | | | | |
|---|----------|----|---------|-------------------------|
| TiO ₂ on perlite granules | Phenol | | UV | (Hosseini et al., 2007) |
| TiO ₂ -SiO ₂ catalyst | Toluene | | UV | (Zou et al., 2006) |
| La-doped TiO ₂ fil | Benzene | | UV | (Zhang et al., 2006) |
| MWNT-TiO ₂ | Phenol | 95 | Visible | (Wang et al., 2005) |
| Ln ³⁺ -TiO ₂ | VOC BTEX | | UV | (Li et al., 2005) |
| TiO ₂ catalyst | Toluene | 46 | UV | (Jeong et al., 2004) |
| Al/TiO ₂ nanometer | Benzene | 60 | UV | (Lee et al., 2003) |
| H ₃ PW ₁₂ O ₄₀ /TiO ₂ /palygorski te | Benzene | | | (Ma et al., 2015) |

2.5.2.2 Heterogeneous catalyst based photocatalytic degradation of petrochemical wastes

Among AOPs, heterogeneous photocatalytic oxidation, which involves the acceleration of photoreaction in the presence of a semiconductor catalyst, has proved to be of utmost interest due to its efficiency in degrading recalcitrant organic compounds. Developed in the 1970s, heterogeneous photocatalytic oxidation has been provided considerable attention by the scientific research community. During the past two decades, numerous studies have been carried out on application of heterogeneous

photocatalytic oxidation processes with an aim to decompose and mineralize recalcitrant organic compounds. Photocatalysts are the class of compounds which generate electron-hole pairs on coming in contact with or on absorption of light quanta. It leads to chemical transformation of substrate that comes in contact with them and regenerates them (Kuen Jo and Tayade 2016). These substances are invariably semiconductors. Semiconductor heterogeneous photocatalysis has enormous potential to treat organic contaminants in water and air.

Many semiconductors have been studied for the degradation of petrochemical compounds. Several semiconductors (TiO_2 , ZnO , Fe_2O_3 , CdS , ZnS) can act as photocatalysts. However, TiO_2 has been most widely and universally studied because of its high reactivity, reduced toxicity, chemical stability, lower costs, its ability to break down organic pollutants and resulting in complete mineralization (Ahmed et al., 2010; Carraro et al., 2014; Hao et al., 2015; Zhu et al., 2010). It shows stability even at low pH. Photocatalytic and hydrophilic properties of TiO_2 make it close to an ideal catalyst. Fujishima and Honda (1972) showed the possibility of water splitting in a photoelectrochemical cell containing an inert cathode and rutile titania anode. The applications of titania photoelectrolysis has since been greatly focused for environmental pollution abatement efforts including water and wastewater treatment (Ahmed et al., 2010; Ren et al., 2015; Sood et al., 2015). In addition to these applications, various other applications of TiO_2 as photocatalyst for the degradation of various environmental pollutants are discussed later.

The general mechanisms involved for degradation of petrochemicals (organic compounds) by titania nanomaterial based photocatalysts are shown in figures 2.8 and 2.9. It revealed that in presence of OH^\bullet radicals, the phenolic compound gets converted into polyphenolic products, which further get converted to aldehydes and carboxylic acids by oxidation and finally get mineralized to CO_2 and H_2O .

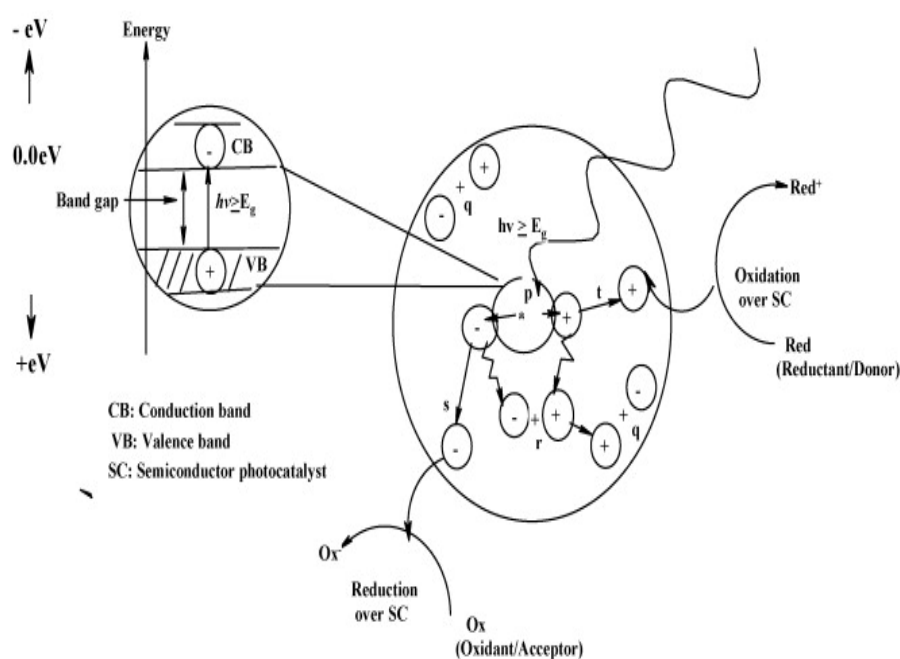


Fig. 2.8: Schematic photophysical and photochemical processes over photon activated semiconductor cluster (p) photogeneration of electron/hole pair, (q) surface recombination, (r) recombination in the bulk, (s) diffusion of acceptor and reduction on the surface (adapted from Gaya and Abdullah 2008, with permission)

2.5.2.2.1 TiO_2 as photocatalysts

Titania, also known as titanium dioxide (TiO_2), is a naturally occurring oxide of transition metal family. It is the most commonly used catalyst in photocatalytic degradation. It has been widely explored than other heterogeneous photocatalysts due

to various electrochemical properties such as low cost, chemical stability, non-toxicity, resistance to photo-induced reaction, degrade a wide range of organic pollutants, etc. (Abdullah et al., 2016; Nasir et al., 2016). It is used in various industries, including cosmetics, medicines, textiles, electronics, pharmaceuticals, and environmental remediation (Feizi et al., 2012). Various studies have been conducted for photodegradation of various pollutants from water using the photocatalytic properties of TiO₂, which are outlined in table 2.5.

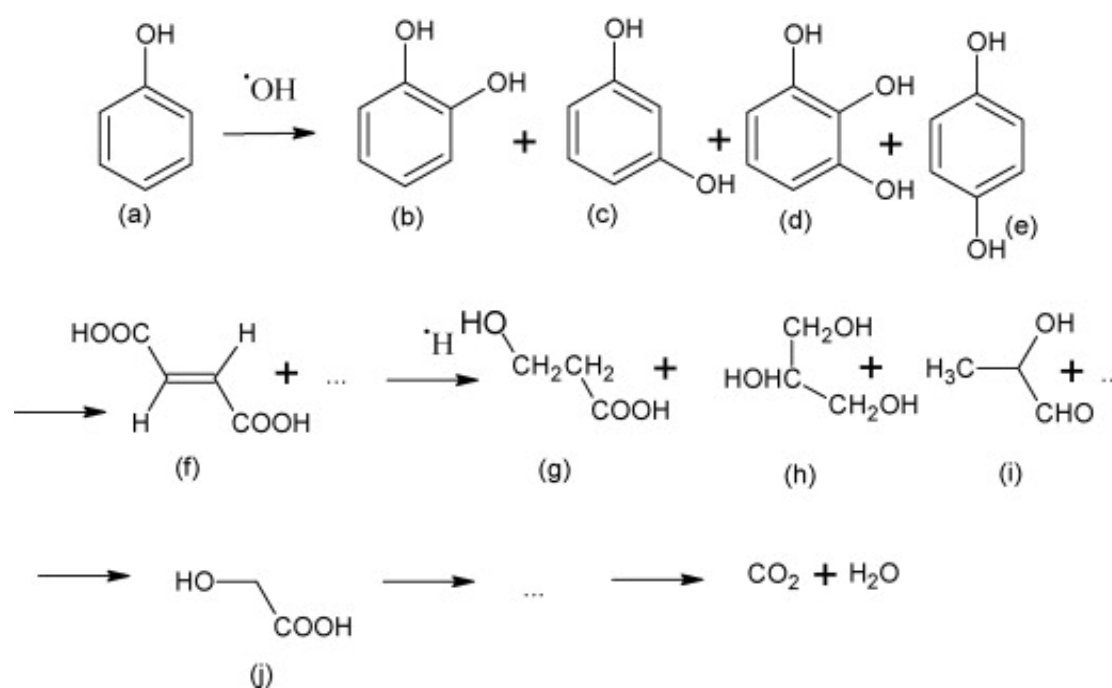


Fig. 2.9: Photocatalytic degradation of phenol in wastewater (adapted from Gaya and Abdullah 2008, with reprinted permission).

2.5.2.2.2 ZnO based photocatalysts

ZnO semiconductors based photocatalysts are widely used for degradation of a wide range of organic pollutant released from the petrochemical waste (e.g., benzene,

xylene, phenol, hexane, naphthalene present in water, air and soil) (Barreca et al., 2007; Ferrari-Lima et al., 2015; Gondal and Sayeed 2007; Hernandez-Garcia et al., 2016; Silva et al., 2014). However, several factors regulate the photocatalytic degradation efficiency of a catalyst. For example, major factors influencing the catalysts photodegradation efficiency can be their structure, particle size, dispersibility, band gap and the hydroxyl density on the surface (Feng et al., 2014; Pardeshi and Patil 2009; Xu et al., 2009). ZnO based catalysts have high sensitivity to light and greater band gap (3.37eV). Thus, it have the ability to absorb in a wider range of solar spectrum than other photocatalysts like TiO₂ (Pardeshi and Patil, 2009). The greater band gap of ZnO helps in better oxidation-reduction activities (Saucedo-Lucero and Arriaga, 2013).

2.5.2.2.3 CdS-based photocatalysts

CdS is also one of the well-studied photocatalysts used for the degradation of organic compounds. It has narrow band gap (2.4 eV) which helps in its greater activity and better degradation potential of various environmental pollutants (Wang et al., 2016b). In addition, it forms hetero-junction composite with other photoactive materials which further improves its photocatalytic activities (Fan et al., 2016). Moreover, CdS has been extensively studied for photodegradation of petrochemical waste along with other semiconductors like TiO₂, AgCl, ZnO as heterogeneous photocatalytic nano-composites (Li et al., 2015c). Composite of the CdS with other semiconductor have higher efficiency for the photo-degradation of organic pollutants (Fan et al., 2016; Gupta and Pal 2014; Hernández-García et al., 2015; Kim and Kan

2015; Meng et al., 2012; Wang et al., 2016b; Wang et al., 2011; Wilson et al., 2012; Zhu et al., 2012). It has almost similar band structure and other photocatalytic properties as that to TiO₂. Thus, CdS/TiO₂ composite has also been extensively studied in several photodegradation studies (Kim and Kan, 2015). Generally, CdS get excited under visible light irradiation, which limits its application in this spectral region. The dual advantage of such composites (CdS/TiO₂) involves a mechanism of photodegradation under which the photoexcited electron in the conduction band of CdS get transferred to the conduction band of TiO₂ leaving behind a hole in the valence band of CdS. Thus, it helps in photodegradation under visible range as well as improves the organic pollutant degradation efficiency of the composite material (Hernández-García et al., 2015).

2.5.2.3 Factors affecting the photo-degradation performances

2.5.2.3.1 Catalyst loading

Various scientific studies and reports showed that the amount of catalyst plays a vital role in the degradation of organic pollutants, mostly in aqueous phase. The amount of catalyst used in the degradation processes is directly proportional to the overall degradation rate of organic compound (Akpan and Hameed 2009; Diyauddin et al., 2011; Jain and Srivastava 2008; Prabha and Lathasree, 2014). However, increasing concentration of catalysts beyond certain limits would not result in any significant change in the rate of degradation processes (Alhakimi et al., 2003), and even decreases light penetration and photoactivated volume by increasing the turbidity of the solution (Akyol et al., 2004). It is also postulated that the excessive

use of catalyst causes light scattering and a screening effect, thus, reduces the specific activity. In this case catalyst surface become unavailable for photon absorption and pollutant adsorption which reduces the reaction rate (Gao et al., 2006; Gaya and Abdullah, 2008). Similar phenomenon has also been reported in several other studies (Heredia et al., 2001; Mehrotra et al., 2003; Saien and Soleymani 2007; Saien et al., 2003). However, in the case of TiO_2 , its excessive use as a catalyst causes the shielding effect.



Here, TiO_2^* is the active species which adsorbs on the surface, while $\text{TiO}_2^\#$ is the deactivated form (Harman, 2005; Tayade et al., 2009). Therefore, to avoid the use of excess catalyst, it is imperative to optimize the amount of catalysts that is actually needed to obtain the required degradation rate (Tayade et al., 2009).

2.5.2.3.2 pH of the solution

Effect of pH of the solution on photocatalytic degradation of organic pollutants and their adsorption on the catalyst surface has been studied by many investigators (Akpan and Hameed, 2009; Alakahami et al., 2003; Mrowetz and Shelli, 2006; Wang and Ku, 2007). pH is an important parameter because of the fact that different effluents need to be treated at different pH values. Further, pH regulates the surface charge properties of the photocatalyst and the size of aggregates they form. For example, the surface of TiO_2 remains positively charged in acidic medium (pH

less than 6.9) and negatively charged in alkaline medium (pH greater than 6.9) due to its amphoteric nature.



Nataranjan et al. (2011) studied the effect of pH in dye degradation and found that when the pH of the solution is decreased from 6.5 to 2.9, the percentage of degradation and decolorization decreases. This must be due to the acidic solution retraining the adsorption of dye. Also, when the pH value of the solution is increased from 6.5 to 9.8, it is observed that up to pH 8.8 of the solution, there is an increase in the percentage of degradation and decolorization.

2.5.2.3.3 Concentration and nature of pollutants

The effect of the concentration and nature of organic pollutants on the rate of photocatalytic degradation has been widely studied (Malekshoar et al., 2014). The organic compounds which have greater tendency to bind to the catalyst surface, such as aromatic hydrocarbons, are more prone to be oxidized (Pardeshi and Patil, 2009). However, it majorly depends on the substituent group of these organic pollutants. Further, the high concentration of pollutants in aqueous medium saturates the catalyst surface, which reduces photonic efficiency, and thus, ultimately causes deactivation of photocatalysts (Lee et al., 2016; Prabha and Lathasree 2014). Therefore, it can be concluded that photocatalytic degradation of organics (aromatic and non-aromatic) depends on the substitution group.

2.5.2.3.4 Light/radiation source for photocatalysis

Photocatalytic degradation techniques require high energy sources. UV radiation and sunlight- both can be used for photocatalytic process; however UV radiation is mostly used. Various recent studies have been conducted on LEDs as light source for photodegradation of organic compounds, mainly in aqueous and air medium (Chen et al., 2007; Daniel and Gutz 2007; Tayade et al., 2009; Wan-Kuen Jo et al., 2014). Photocatalysis reactions depend mainly on the radiation absorption by catalysts, which, in turn, relies on the intensity of light. The photon flux enhances the degradation due to favourable collision chances between photon and activated centre (Silva et al., 2007; Wang et al., 2007). Various other studies also support that an increase in intensity of light leads to an increase in the degradation rate of organic pollutants (Karunakaran and Senthilvelan 2005; Qamar et al., 2006). The nature of light does not affect the pathway of reaction (Gaya and Abdullah 2008).

2.5.2.3.5 Reactors used in photocatalytic degradation

Photocatalytic reactors can be classified based on the deployed state of the photocatalyst, i.e., by whether it is suspended or attached. Photocatalytic reactors can use either UV or solar radiation. Solar photocatalytic reactors have been of great interest for the photo-oxidation of organic contaminants in water (Petit et al., 2007; Sarkar et al., 2015; Sia et al., 2015; Vincent et al., 2009; Yao and Kuo 2015). Hybrid photocatalytic membrane reactors have been developed to achieve the purpose of downstream separation of photocatalyst (Guo et al., 2015; Molinari et al., 2015a; Molinari et al., 2015b; Molinari et al., 2014; Molinari et al., 2013; Mozia 2010; Mozia

et al., 2014; Ong et al., 2014). Membrane photoreactors appear to be a promising alternative to conventional photoreactors and more research in this area can assist to overcome some of the problems faced with the use of conventional reactors (Khan et al., 2015; Motamed Dashliborun et al., 2013; Mozia et al., 2014; Mozia et al., 2013; Nasir et al., 2016).

With majority of treatment techniques having major drawback of generation of another type of wastes, biodegradation and photocatalytic mineralization looks a very promising techniques. Bioremediation is proved to be a not only cost-effective technique; it also leads to complete mineralization of the organic pollutants and generates nontoxic end products. It is undoubtedly a sustainable and economically feasible technique for the remediation of environmental pollutants. , photocatalysis is also one of the most promising techniques for the degradation of wide range of organic pollutants in liquid and gas forms. Another unexplored areas of the degradation of organic pollutant is to devise and promote hybrid processes (based on both the bioremediations and photocatalytic degradation routes) of two above mentioned techniques.
