of different types of organic and inorganic pollutants, and it is especially useful for the treatment of recalcitrant pollutants (such as AHCs, xenobiotics, and pharmaceuticals) (Hassard et al., 2014; Pakshirajan and Singh, 2010). RBC's principal advantages are low space requirement, easy construction, short hydraulic retention time (HRT), and high biomass concentration per unit volume of the reactor (Cortez et al., 2008; Hassard et al., 2014). The pre-treatment requirement, high energy cost due to the continuous rotation of the shaft, and limited process flexibility are the demerits of this process. The biodegradation of Direct Red-80 and Mordant Blue-9 dye was studied in RBC, and found that the RBC could remove more than 90% of each dye within 24 hours (Pakshirajan and Singh, 2010).

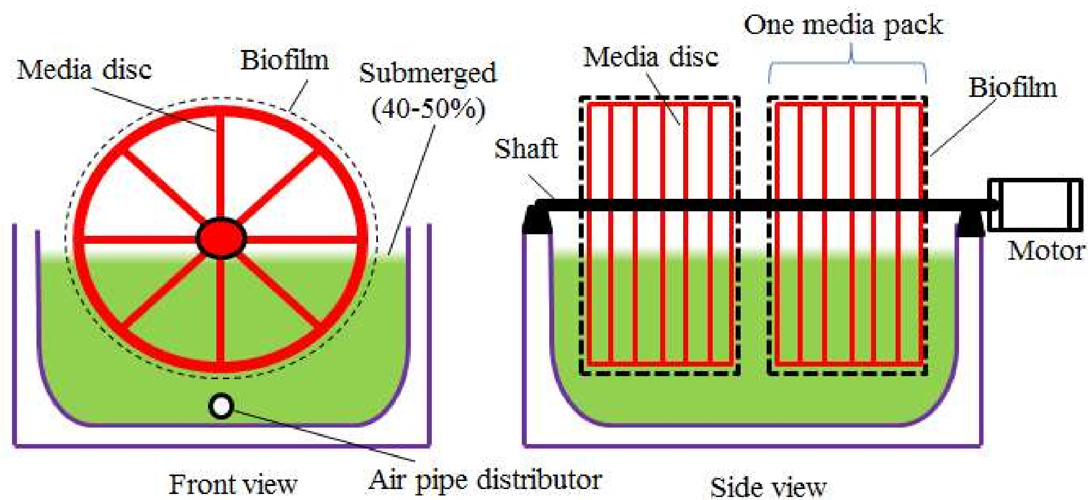
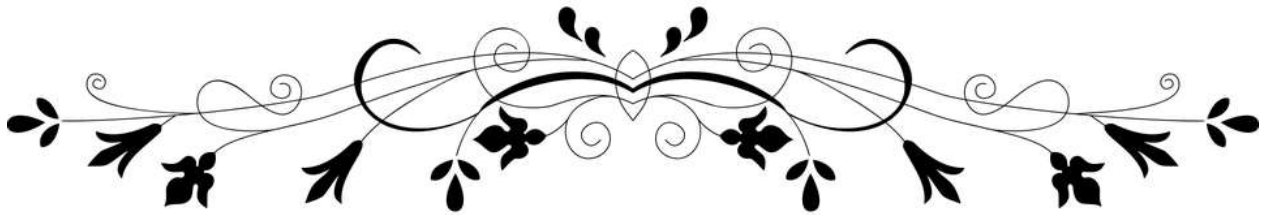


Figure 1.8. A schematic diagram of a rotating biological contactor.



CHAPTER 2

Literature Review

CHAPTER 2

Biodegradation has been employed extensively for the removal of dyes and polycyclic aromatic hydrocarbons (PAHs) in terrestrial and aquatic ecosystems (Umar et al., 2017; Varjani and Upasani, 2017a). In biodegradation, the microorganisms metabolize pollutants as their energy sources (Biswas et al., 2015). Biodegradation of dyes and PAHs via bacteria, fungi, and algae are described in the following subsections.

2.1. Biodegradation of dye *via* bacteria, fungi, and algae

2.1.1. Biodegradation of dye *via* bacteria

Bacteria are the class of microorganisms that are able to degrade different types of dyes under aerobic or anaerobic conditions. Previously, different groups of bacterial species, namely *Bacillus* sp. MH587030.1 (Sonwani et al., 2020a), *Photobacterium leiognathi* strain MS (Sutar et al., 2019), *Bacillus subtilis* (Shalini and Setty), *Cedecea davisae* (Cao et al., 2019), *Alcaligenes faecalis* (Bharti et al., 2019), *Lysinibacillus* sp. RGS (Saratale et al., 2013), *Aeromonas hydrophila* (Bouraie and Din, 2016), etc., have been successfully used in the biodegradation of various dyes.

The biodegradation of dye can take place under both aerobic and anaerobic conditions by bacterial strains. In both conditions, the reduction of azo bonds (–N=N–) is the initial step in the biodegradation of dye. The reduction may involve different mechanisms, including enzymatic reduction and low molecular weight redox mediators, and the location of these reactions may be either intracellular or extracellular sites (Singh et al., 2015). Azo dyes are difficult to degrade under aerobic conditions because of the presence of oxygen, which generally repels the azo bond reduction activity (Ayed et al., 2011; Kodam et al., 2005; Ola et al. 2010). However, several bacterial species

have been found to be more effective in dye degradation, and these were enriched and isolated from dye contaminated soil, sediment, wastewater, and activated sludge (Bharti et al., 2018; Kodam et al., 2005; Padmanabhan et al., 2016; Solis et al., 2012). Most of these species need organic carbon sources (e.g., glucose, fructose, etc.), as it is difficult to utilize dye as a sole growth substrate. However, several isolated bacterial species were found to be effective in the biodegradation of dye in the presence of organic carbon sources (Bharti et al., 2019; Solis et al., 2012; Sonwani et al., 2020a; Talha et al., 2018). For example, an isolated bacterial species (*Bacillus* sp. MH587030.1) was able to decolorize the Congo red dye in the presence of glucose under aerobic condition (Sonwani et al., 2020a). In another work, *Micrococcus* sp. was successfully used to decolourise the reactive dyes under aerobic conditions within 6 h (Olukanni et al., 2009). Nachiyar and Rajkumar (2003) have also studied the biodegradation of a Navitan Fast blue S5R dye by *Pseudomonas aeruginosa*. The bacterial species (e.g., *Alcaligenes faecalis*, *Pigmentiphaga kullae* K24) were able to grow in the presence of azo dye and utilized them as the source of carbon and energy (Bharti et al., 2018; Pandey et al., 2007).

Generally, the bacterial degradation of azo dyes involves the reductive cleavage of azo bonds (–N=N–) by azoreductase under anaerobic condition. This results in dye decolonization and the formation of colorless solutions containing potentially hazardous-aromatic amines (Chang and Kuo, 2000, Chang et al., 2004). The obtained intermediates are further subjected to degradation by aerobic process to achieve the complete mineralization of dye. The formation of intermediates aromatic amines are the major limitations of the anaerobic process. However, these intermediates are further broken down into simpler compounds by an aerobic treatment.

2.1.2 Biodegradation of dye *via* Fungi

Fungi is a type of microorganism and widely used in textile effluent treatment (Singh et al., 2015). Fungi reveal more physical and enzymatic contact with pollutants due to their higher cells to surface ratio. The literature survey reveals that the white-rot fungi are most effective in the biodegradation of synthetic dyes (Ali et al., 2010). The white-rot fungi such as *Phanerochaete chrysosporium, Trametes versicolour,* and *Coriolus versicolour* have also been used for the removal of dyes (Chander and Arora, 2004; Masud and Anantharaman, 2006). Similarly, non-white-rot fungi like *Aspergillus niger* and *Cunninghamella elegans* have also been tested in the removal of dyes (Ambrosio and Campos-Takaki, 2004; Fu and Viraraghavan, 2002). Mahmoud et al. (2017) have reported that the *Aspergillus Niger* decolorized 1000 mg/L of red azo dye at pH of 9.0. According to Solis et al. (2012), filamentous fungi are abundantly available in the environment, and its application in dye removal is an attractive option due to the low cost and the possibility of complete dye mineralisation. The removal of dye by fungi is based on biosorption, biodegradation, and bioaccumulation mechanisms. The adsorption capacity (AC) of the fungal biomass increased with increasing process temperature, which could probably be due to the high surface energy (Kaushik and Malik, 2009). However, the decolorization efficiency decreased at very high temperature due to the deactivation of the adsorbent surface.

2.1.3 Biodegradation of dye *via* Algae

Algae is a type of photosynthetic organism and can be obtained in nearly all parts of the world (Khan et al., 2013). In the last few decades, the application of algae has received worldwide attention in the wastewater treatment. The algae strains, such as *Chlorella vulgaris, Scenedesmus quadricauda, Chlorella ellipsoidea, Chorella kessleri, Oscillatoria rubescens, Chlorella pyrenoidosa,* and *Phormidium autumnale* UTEX158*0*

have been used in the biodegradation of dyes (Vikrant et al., 2018; Solis et al., 2012). The removal of dye by algae is mainly due to three mechanisms; assimilation of chromophores by photosynthesis, the conversion of coloured molecules into non-coloured ones, and adsorption phenomena (Khan et al., 2013). Different species of *Chlorella* and *Oscillitoria* are able to breakdown the azo bond to their intermediate aromatic amines, which is further metabolized into simpler organic compounds or CO_2. Youssef et al. (2008) have reported the biodegradation of malachite green using *Acremonium kiliense,* and they found that 95.4% of dye removal was achieved in 72 h. Sarnthima et al. (2009) also studied the removal of different dyes, namely Indigo carmine, Bromophenol blue, Methyl red, and Remazol brilliant blue using *Lentinus polychrous* and found satisfactory results within 16 h.

2.2. Review on biodegradation of dye

Gopinath et al. (2009) have studied the biodegradation of Congo red dye using *Bacillus* sp. The process variables were optimized and the maximum removal of dye was obtained at pH 7.0 and temperature of 37 °C. Also, the dye removal efficiency was the highest at the lowest concentration of Congo red. Further, as the dye's concentration increased, the dye removal efficiency was significantly decreased, and the time required for the degradation was increased. The obtained experimental data were analyzed using Monod growth model and Haldane substrate inhibition model. Parshetti et al. (2010) have used *Kocuria rosea* (MTCC 1532) for the biodegradation of Methyl orange. The strain was capable to completely remove 50 mg/L of Methyl orange under optimum pH (6.8) and temperature (30 °C). The reductase i.e., NADH-DCIP reductase and azoreductase, were responsible for the biodegradation of Methyl orange. Phytotoxicity and microbial toxicity study demonstrated that Methyl orange was toxic, and

intermediates metabolites obtained after its decolourization was non/less toxic for *Triticum aestivum* and *Phaseolus mungo* seeds.

Du et al. (2013) studied Malachite green degradation using isolated *Micrococcus* sp. BD15 from sewage. The experiments were performed with 100 mg/L of Malachite green at 30 °C and 200 rpm. They reported that the intermediate metabolites; 4-(Dimethylamino) benzophenone, Michler's ketone, 4-(methylamino)benzophenone, 4-aminobenzophenone, 4- methylaminobenzoic acid, 4-hydroxyl-N,N-dimethylaniline, N,N-dimethylaniline, hydroxyl-4 (dimethylamino) benzophenone and 4-hydroxyl-aniline were formed during the biodegradation of Malachite green. The biodegradation of three dyes, namely Remazol black, Methyl orange, and Benzyl orange was investigated with sol-gel immobilized *Pseudomonas* sp. (Tuttolomondo et al., 2014). The immobilized *Pseudomonas* sp. was able to produce more than seven times improved amounts of extracellular enzymes. The complete removal of these dyes up to 50 mg/L were obtained within 48 h. However, as the concentration of dyes increased to 75 and 100 mg/L, the immobilized *Pseudomonas* sp. could decolorize nearly 90% after 48 h. The reusability of the immobilized *Pseudomonas* sp. was also analysed with repeated batch experiments, and the decolourization of 75%, 79%, and 83% was obtained for remazol black, methyl orange, and benzyl orange, respectively. The immobilized system was an effective and economical approach for the biodegradation of dye. In another study, Agrawal et al. (2014) have studied the degradation of Acid black 210 dye by *Providencia* sp. SRS82, isolated from soil and wastewater sample of textile industries located near Indore, India. The optimum conditions of temperature, pH, NaCl concentration, and inoculum were obtained at 30 °C, 8.0, 0.25 g/L, and 8×10^8 CFU/mL, respectively. *Providencia* sp. SRS82 could completely decolorize 100 mg/L of Acid black 210 dye within 90 min, under optimum conditions. It can also survive at a

very high concentration (2000 mg/L) of Acid black 210. The toxicity studies indicated that the treated effluent was less harmful than the untreated dye.

Lade et al. (2015) have examined the biodegradation of Congo red dye and real textile effluent using microbial consortium isolated from a soil sample collected from nearby textile processing units. The polyurethane foam immobilized consortium exhibited complete removal of Congo red dye within 12 h, at pH of 7.5 and temperature of 30 ± 0.2 °C. Under similar conditions, 50% of textile effluent was removed within 20 h. The reduction of total organic carbon (83% and 79%), COD (85 and 83%), and biochemical oxygen demand (79 and 78%) were obtained for Congo red dye and textile effluent, respectively. Padmanaban et al. (2016) have evaluated the biodegradation of Reactive red 120 using free cells and PUF immobilized *Bacillus cohnii RAPT1*, isolated from seawater of Tuticorin coast, Tamilnadu, India. The process parameters such as dye concentration, process time, inoculum size, pH, and temperature were optimized in batch mode and optimum conditions were obtained as 200 mg/L, 36 h, 3.0×10^{8} CFU/mL, 8.0, and 35 °C, respectively. The complete removal of Reactive red 120 was obtained under optimum conditions. The dye removal efficiency was significantly higher with PUF immobilized *Bacillus cohnii RAPT1* than free cells. Also, the biodegradation dye followed the second-order kinetics.

Krishnan et al. (2017) studied the biodegradation of three dyes, namely reactive brilliant red X-3B, direct blue-6, and direct black-19 using mixed microbial culture. The effects of process variables such as pH (5.0-9.0), inoculum dosage (5-15% v/v), and initial dye concentration (20-100 mg/L) were studied. The optimum removal efficiencies of brilliant red X-3B, direct blue-6, and direct black-19 were found as 31.2%, 71.5%, and 87.6%, respectively. The kinetics of dyes degradation were studied using Monod and substrate inhibition model. Das and Mishra (2017) have investigated

the biodegradation of Reactive green-19 dye in aqueous solution using the consortium of *Zobellella taiwanensis* (FJ999669.1) and *Bacillus pumilus* (KJ741252.1). The process variables such as temperature, pH, and Yeast extract concentration were optimized using Box Benkan design (BBD) of response surface methodology (RSM). The optimum conditions of temperature, pH, and Yeast extract concentration were obtained to be 32.0 °C, 8.3, and 0.116 g/L, respectively. At optimum conditions, more than 95% of dye removal was obtained within 24 h. The biodegradation of dye followed the first-order rate kinetics. In another work, Elfarash et al. (2017) used two isolated strains i.e., *Pseudomonas aeruginosa* (ASU3 and ASU 6) for the biodegradation of Disperse Blue 64 and Acid Yellow 17. The strain ASU3 and ASU6 were able to degrade 61.2% and 96.8% of Disperse Blue 64, respectively. Furthermore, ASU3 and ASU6 could degrade 38.6% and 91.2 % of Acid Yellow 17 dye, respectively, at the same time. The phytotoxic analysis of untreated and treated water indicates that the treated water reveals more germination *triticum aestivum* than untreated wastewater.

Haq et al. (2018) have examined the biodegradation of Azure-B dye using *Serratia liquefaciens.* The process variables, namely pH (5.0-9.0), agitation speed (100-140 rpm), and temperature (25-35 °C), were optimized using BBD of RSM. More than 90 % of dye removal was obtained at optimum conditions (pH= 7.0, temperature= 30 °C, and agitation= 120 rpm). *Vigna radiata* seeds germinated in treated wastewater indicated improved growth than untreated wastewater. Hameed and Ismail (2018) have studied the biodegradation of Reactive Red 2 dye in a sequential anaerobic-aerobic bioreactor. The mixed culture obtained from a local sewage treatment plant was able to completely decolorize 10 mg/L of Reactive Red 2 dye within 30 h. The *triticum aestivum* seeds grown in treated sample showed 100% germination, which is much higher than the Reactive Red 2 -loaded solution (only 30% germination).

The polyurethane foam immobilized *Bacillus subtilis* was used to degrade the Congo red dye (Shalini and Shetty, 2019). They optimized the process parameters such as pH, glucose concentration, and Congo red concentration, and the optimum conditions were obtained as 8.0, 4.0 g/L, and 50.0 mg/L, respectively, with 92% of dye removal efficiency. In the biodegradation of synthetic and real textile wastewater, more than 85% of COD and 55% of total organic carbon were reduced in both types of wastewater. The active surface area of carrier and biological interaction are vital factors that affect the immobilization of microorganisms. Cao et al. (2019) have reported the removal of crystal violet by *Cedecea davisae*, isolated from sludge samples of the aerobic reactor. At optimum conditions (pH 7.0, temperature 30 °C, and crystal violet concentration 40 mg/L), *Cedecea davisae* was able to decolorize more than 95% of crystal violet and reduced 90% of COD within 48 h. Bharti et al. (2019) have isolated *Alcaligenes faecalis* isolated from the carpet industry located near, Bhadohi, Varanasi and used for the biodegradation of Methylene blue dye in a packed bed bioreactor (PBBR). The process parameters such as inoculum size, temperature, agitation, and process time were optimized and obtained as 3 mL, 30 °C, 150 rpm, and 5 days, respectively. The maximum removal of Methylene blue (96.2%) was found under optimum conditions. Furthermore, the performance of a recycled PBBR packed with bio-char immobilized *Alcaligenes faecalis* was evaluated, and it was found that PBBR successfully removed 87 % of dye at the initial concentration of 500 mg/L. The immobilized cells were able to withstand at higher concentrations of dye than free cells. The decolourization of Methylene blue was followed by the pseudo-second order kinetics. A summary of microbial species used in the biodegradation of dyes, analytical techniques, and their metabolites is reported in **Table 2.1**.

Table 2.1 A summary of microorganisms and process conditions used in the biodegradation of dyes.

S.N.	Dye	Microorganism	Process condition (Batch study)				Removal efficiency	Reference
			Conc. (mg/L)	pH	Temperature (°C)	Time (h)		
1	Malachite green	*Photobacterium leiognathi* strain MS	1000	8.0	30	24	92.5	Sutar et al. (2019)
2	Congo red	*Bacillus subtilis*	100	8.0	37	24	92	Shalini and Setty (2019)
3	Malachite green	*Bacillus cereus M 16*	50	7.0	30	72	96	Nath et al. (2019)
4	Naphthol green B	*Pseudoalteromonas sp. CF10-13*	500	7.5	25	80	>95%	Cheng et al. (2019)
5	Crystal violet	*Cedecea davisae*	40	7.0	30	48	>95	Cao et al. (2019)
6	Methylene Blue	*Alcaligenes faecalis*	50	7.0	30	120	81.5	Bharti et al. (2019)
7	Azure-B	*Serratia liquefaciens*	100	7.6	30	48	90	Haq et al. (2018)
8	Reactive red 2	*Consortium*	20	7-7.6	30±3.0	30	100	Hameed and Ismail (2018)
9	Reactive green 19	*Zobellella taiwanensis, Bacillus pumilus*	100	8.3	32.0	24	95	Das and Mishra (2017)
10	Reactive red 120	*Bacillus cohnii RAPT1*	200	8.0	35	36	100	Padmanaban et al. (2016)
11	Reactive black 5	*Aeromonas hydrophila*	100	7.0	35	24	76	Bouraie and Din (2016)
12	Congo red	Consortium	100	7.5	30	12	100	Lade et al. (2015)

13	Acid black 210	*Providencia* sp. SRS82	100	8.0	30	1.5	100	Agrawal et al. (2014)
14	Remazol red	*Lysinibacillus* sp. RGS	50	7.0	30	6.0	>90	Saratale et al. (2013)
15	Rhodamine B	*Coelastrella sp.*	100	8.0	30	480	80	Baldev et al. (2013)
16	Naphthol green B	*Shewanella oneidensis* MR-1	1000	8.0	30	24	90>	Xiao et al. (2012)
17	Orange 2	*Pseudomonas putida SKG-1*	100	8.0	30	96	92.8	Garg et al. (2012)
18	Methyl orange	*Kocuria rosea* (MTCC 1532)	50	6.8	30	72	100	Parshetti et al. (2010)
19	Methyl orange	Consortium *(Sphingomonas paucimobilis, Bacillus cereus ATCC14579, Bacillus cereus ATCC11778)*	750	7.0	30	48	>90	Ayed et al. (2010)
20	Congo red	*Bacillus* sp.	500	7.0	37	40	100	Gopinath et al. (2009)
21	Malachite green	*Pseudomonas pulmonicola* YC32	50	7.0	35	3.5	85.2	Chen et al. (2009)
22	Direct Blue-6	*Pseudomonas desmolyticum NCIM 2112*	50	7.0 - 7.9	30	72	100	Kalme et al. (2007)

2.3. Biodegradation of PAHs via bacteria, fungi, and algae

2.3.1. Biodegradation of PAHs via bacteria

Bacteria are a class of microorganisms that can actively break down or mineralize aromatic hydrocarbons like PAHs. Many bacterial species, namely *Bordetella avium, Pseudomonas veronii, Klebsiella* sp., *Cronobacter sakazakii*, *Gordonia alkaliphila, Janibacter anophelis* strain JY11, and *Escherichia coli* were used for the biodegradation of PAHs (EI-Naas et al., 2014; Haritash and Kaushik, 2009; Sonwani et al., 2019a). Biodegradation of AHCs occurs through either aerobic or anaerobic process. In aerobic biodegradation, oxygen acts as a final electron acceptor to achieve hydroxylation of the aromatic ring. In contrast, anaerobic biodegradation of AHCs employs diverse approaches mainly based on reductive reactions to breakdown aromatic rings (Ghosal et al., 2016). Bacteria produce enzymes that can drive the breakdown of aromatic rings. Monohydroxylation of aromatic rings occurs due to the activation of monooxygenases, whereas dihydroxylation proceeds with the aid of dioxygenases.

Generally, bacteria favor the aerobic degradation of PAHs by either monooxygenases or dioxygenases. Initially, PAHs are degraded by hydroxylation of an aromatic ring via dioxygenases with the generation of *cis*-dihydrodiol (Ghosal et al., 2016). This *cis*-dihydrodiol is aromatized into a diol intermediate, which further cleaved to extradiol or intradiol ring-cleaving dioxygenases through an *ortho*- or *meta*-cleavage pathway. Such cleavage results in the formation of intermediates (e.g., catechol) and final transformation into tricarboxylic acid (TCA) cycle intermediates (Ghosal et al., 2016; Mallick et al., 2011). During hydroxylation of PAHs, the C-C bond gets weakened and the molecule is transformed into a form that is amiable to cleavage. Cleaving of all rings leads to acetate formation, which enters the Krebs cycle to achieve

complete mineralization into carbon dioxide and water (Haritash and Kaushik, 2009). A proposed metabolic pathway of naphthalene biodegradation by bacteria is shown in **Figure 2.1**. Initially, dioxygenases attack the aromatic ring to broke down it into *cis*-1,2-dihydroxy-1,2-dihydronaphthalene, which is subsequently metabolized into 1,2-dihydroxynaphthalene by *cis*-dihydrodiol dehydrogenases. Successive ring cleavage of 1,2-dihydroxynaphthalene leads to salicylate formation. In addition, the non-enzymatic side reactions oxidize 1,2-dihydroxynaphthalene into 1,2-naphthaquinone. Salicylate is decarboxylated into catechol, which is further broken down by ring fission via the *meta*- and *ortho*-cleavage pathways. A summary of microorganisms used in the biodegradation of PAHs, analytical techniques, and their metabolites are given in **Table 2.2**.

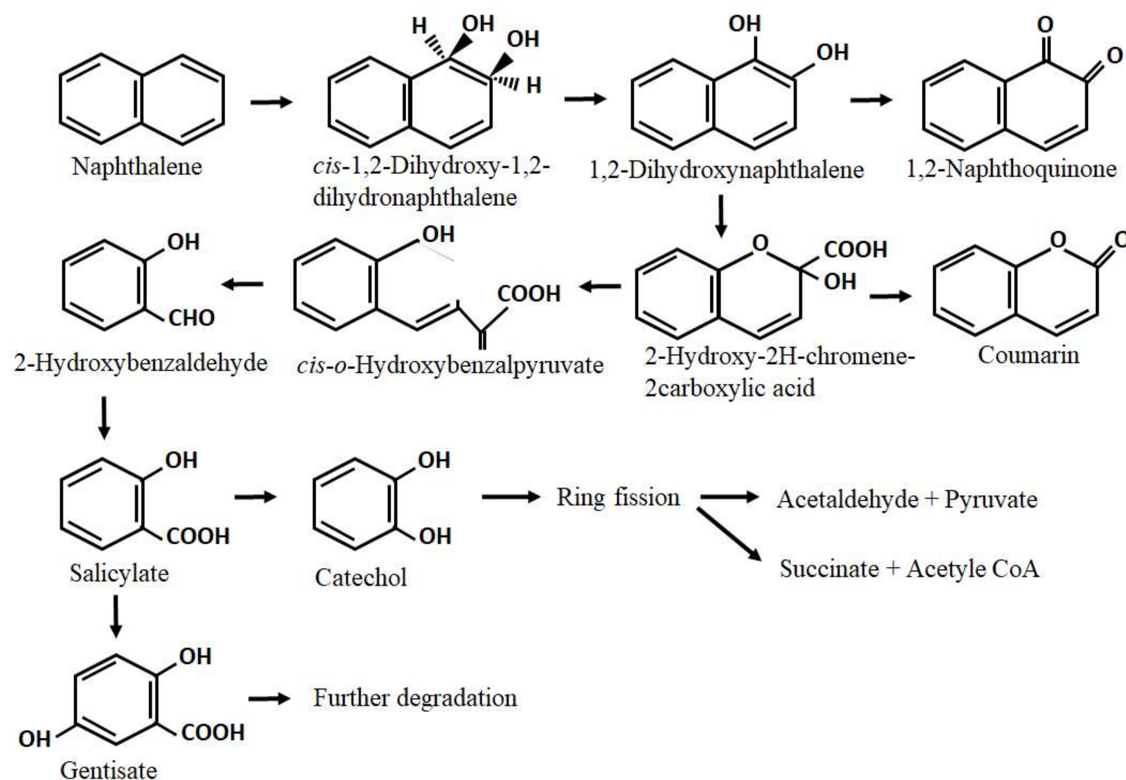


Figure 2.1. Proposed biodegradation pathway of naphthalene (Reproduced from Seo et al., 2009)

2.3.2. Biodegradation of PAHs *via* fungi

Enormous research efforts have focused on the biodegradation of AHCs by fungi (Varjani, 2017). Similar to bacteria, fungi could not utilize PAHs as the sole carbon source; however, fungi may break down AHCs into a number of oxidized products (Ghosal et al., 2016). For example, the biodegradation of naphthalene using *Mycobacterium* sp. is achieved as dioxygenase and monooxygenase, whereas the biodegradation of aromatic hydrocarbons by fungi mainly involves monooxygenases (El-Naas et al., 2014). Silva et al. (2009) reported that fungi, namely, *Aspergillus* sp., *Trichocladium canadense*, and *Fusarium oxysporum*, can biodegrade low molecular weight (LMW)-PAHs, whereas *T. canadense*, *Aspergillus* sp., *Verticillium* sp., and *Acremonium* sp. can biodegrade high molecular weight (HMW)-PAHs under low oxygen levels. The biodegradation of PAHs initiates with the formation of dihydrodiol by dioxygenases. Later, the dihydrodiol breaks down into intermediates, such as protocatechuate and catechol, by either ortho- or meta-pathway (Aydin et al., 2017). Generally, two types of fungi, namely ligninolytic/white-rot fungi (i.e., produce lignin peroxidases, manganese peroxidases, and laccases) and non-ligninolytic fungi (i.e., produce cytochrome P450 monooxygenase-like enzymes) have been used for the biodegradation of AHCs (Ghosal et al., 2016; Tortella et al., 2005).

2.3.3 Biodegradation of PAHs *via* algae

Diverse algae strains, such as *Ulva prolifera, Oscillatoria* sp., *Selenastrum capricornutum*, *Sphingomonas yanoikuya*e, *Chlorella sorokiniana*, *Chlorella vulgaris*, and *Scenedesmus platydiscus*, have been employed for the biodegradation of PAHs (Haritash and Kaushik, 2009; Zhang et al., 2019). DO is necessary in the aerobic biodegradation of PAHs by algae for both activation and cleavage of the benzene ring, and DO acts as the electron acceptor for complete mineralization (El-Naas et al., 2014).

According to Borde et al. (2003), algal–bacterial consortia were applied to breakdown the phenol and phenanthrene. They reported that the *Pseudomonas migulae* and *Sphingomonas yanoikuyae* were effectively degraded the phenanthrene than phenol. Recently, Zhang et al. (2019) reported that *Ulva prolifera* completely removed the PAHs at the initial concentration of 10 μg/L. They revealed that the removal efficiency of PAHs by *Ulva prolifera* was highly pH dependent. Similarly, Arias et al. (2017) described the biodegradation of phenanthrene, fluoranthene, and pyrene by the marine alga i.e., *Rhodomonas baltica*. They observed that the *Rhodomonas baltica* could survive under PAH stress, and removed up to 70% of phenanthrene, fluoranthene, and pyrene.

2.4. Review on biodegradation of PAHs

The potential bacterial species were isolated from PAHs-contaminated farmland in Wuxi, Jiangsu Province, China, and used in the biodegradation of PAHs (Zeng et al., 2010). Two isolated strains of *Mycobacterium* (NJS-1 and NJS-P) were able to biodegrade five PAHs, such as pyrene, phenanthrene, fluoranthene, anthracene, and benzo[a]pyrene. Zhong et al. (2011) have reported the biodegradation of three PAHs, named as phenanthrene, fluoranthene, and pyrene by the mixed culture of *Mycobacterium* sp. and *Sphingomonas* sp. The set of batch experiments were performed in the conical flask with 10 mg/L of an initial concentration of each PAHs at 30 °C and 7.0 pH. The complete removal of phenanthrene was obtained within 3 days, whereas the removal of fluoranthene and pyrene were found to be 71.2% and 50% within 7.0 days. Gas chromatography–mass spectrometry (GC-MS) analysis of degraded samples indicated that eight, six, and two intermediates of phenanthrene, fluoranthene, and pyrene, respectively, were obtained.

Ma et al. (2012) have explored the potential of *Pseudomonas* sp. JM2 for the biodegradation of fluorene and phenanthrene, which was collected from the active sewage sludge of a chemical plant in Jilin, China. The effect of process parameters such as pH and temperature were studied on the biodegradation fluorene and phenanthrene. The optimum pH and temperature were obtained as 6.0 and 37 °C, respectively for the maximum removal of PAHs. The efficacy of *Pseudomonas* sp. JM2 was also tested at very low temperature (4.0 °C), which revealed that *Pseudomonas* sp. could be used to biodegrade the PAHs in winter or very cold regions. The biodegradation of naphthalene was studied with free cells (i.e., *Bacillus fusiformis*) and alginate–polyvinyl alcohol (PVA)–clays beads immobilized *Bacillus fusiformis* (Lin et al., 2014). The removal efficiency of naphthalene with immobilized *Bacillus fusiformis* was much higher than that of using free cells. The immobilized cells showed almost complete removal of naphthalene. The immobilized beads were reusable in nature, and it was found that 94.3% of naphthalene removal obtained up to the eighth cycle. Scanning electron microscopy (SEM) analysis of the immobilized beads showed that the *Bacillus fusiformis* was uniformly distributed within them.

Xu et al. (2016) have studied the biodegradation of two PAHs, named as phenanthrene and fluoranthene by two bacterial strains *Sphingomonas* sp. (PJ1) and *Klebsiella* sp. (PJ2), isolated from the sediment samples, Yangtze River, China. *Sphingomonas* sp. (PJ1) was more efficient in the biodegradation of phenanthrene and fluoranthene than *Klebsiella* sp. (PJ2). The maximum biodegradation of phenanthrene and fluoranthene by *Sphingomonas* sp. (PJ1) were obtained as 74.32% and 58.18 %, respectively. Zeng et al. (2017) examined the biodegradation ability of *Mycobacterium* sp. NJS-P for the biodegradation of benzo[a]pyrene. Ring-hydroxylating dioxygenases (RHDs) play a major role in the biodegradation of benzo[a]pyrene. *Mycobacterium* sp.

NJS-P could remove more than 90% of benzo[a]pyrene within 24 h. Umar et al. (2017) have reported the biodegradation of phenanthrene and pyrene by *Cronobacter sakazakii* MM045 (KT933253), isolated from the engine oil-contaminated soil. The process variables such as agitation, temperature, pH, inoculums volume, and salinity were optimized using central composite design (CCD) of RSM. *Cronobacter sakazakii* was able to completely remove phenanthrene and pyrene within 24 h. The highly acidic or alkaline condition imposed an adverse effect on the biodegradation efficiency of *Cronobacter sakazakii*, whereas neutral pH was favourable for the maximum removal of phenanthrene and pyrene. GC-MS confirmed the intermediates such as pyrene cis-4,5-dihydrodiol, 3,4-dihydroxy phenanthrene, phthalate, pyruvic acid, oxalic acid, lactic acid, and acetic acid, were formed during biodegradation of phenanthrene and pyrene.

In another study, the biodegradation of naphthalene was studied with *Bordetella avium*, isolated from wastewater of petroleum refinery in Egypt (Abo-State et al., 2018). Five species, namely MAM-P9, MAM-P14, MAM-P22, MAM-P25, and MAM-P26 showed naphthalene biodegradation potential. Among these isolates, MAM-P22 showed the best results in the biodegradation of naphthalene, which further confirmed as *Bordetella avium* (MAM-P22) by 16S-rRNA sequencing. *Bordetella avium* could remove 98% of naphthalene, and six intermediates were formed after its degradation. Liu et al. (2019) have studied the biodegradation of phenanthrene by immobilized *Bacillus* sp. P1 in the presence of various Cd (II) concentration. The materials like polyvinyl alcohol-sodium alginate (PVA-SA) and PVA-SA-cell cryogel beads were prepared and used for the immobilization *Bacillus* sp. P1 cells. The phenanthrene removal was considerably increased with immobilized beads due to the increase of the adsorption site. The maximum phenanthrene removal (88%) was found at pH 7.0, while the lowest removal (69.8%) was obtained at pH 4.0. The solubility of phenanthrene was

increased with increasing temperature, which enhanced the bio-availability. Initially, phenanthrene removal increased with increasing temperature from 20 to 30 °C, and the removal was decreased beyond this. At very high temperature, the enzymatic activity could be inhibited, and corresponding reduced the phenanthrene removal. Xu et al. (2019) have studied the biodegradation of three PAHs, namely phenanthrene, fluoranthene, and pyrene by isolated *Klebsiella* sp. from soil washing effluent. *Klebsiella* sp. was immobilized with polyvinyl alcohol (PVA)-sodium alginate (SA)-nano alumina (ALNPs) composite, and used in the biodegradation PAHs. PAHs removal efficiency with immobilized *Klebsiella* sp. was higher than that of suspended cells.

Table 2.2. A summary of microorganisms used in the biodegradation of PAHs.

S.N.	PAHs	Microorganism	Process condition (Batch study)				Removal efficiency	Reference
			Conc. (mg/L)	pH	Temperature (°C)	Time (Day)		
1	Phenanthrene, Fluoranthene, Pyrene	*Klebsiella* sp.	20	7.0	25	7.0	89.14 % PHN, 81.25% FLT, 78.33% PYR	Xu et al. (2019)
2	Phenanthrene	*Bacillus* sp. P1	60	4.0-9.0	20-50	2.0	92.05% PHN with free cell & 98.62% PHN with immobilized cell	Liu et al., 2019
3	Naphthalene	*Bordetella avium*	5	7.0	30	7.0	95% at 5 mg/L	Abo-state et al. (2018)
4	Benzo[a]pyrene	*Cellulosimicrobium cellulans* CWS2	10	3.0-12	20-35	13	78.8% at 10 mg/L	Qin et al. (2017)
5	Phenanthrene, Pyrene	*Enterobacter* sp. MM087	50-200	7.0	30	17	100% PHN, PYR	Umar et al. (2017)
6	Phenanthrene , Naphthalene, Fluoranthene, Pyrene	*Achromobacter* sp . LH-1	100	7.0	30	5.0	93.3% PHN, 83.5% NAP, 70.6% FLT, 25% PYR	Hou et al. (2018)

7	PYR	*Pseudomonas* sp. JPN2	100	7.0	30	22	82.8%	Jin et al. (2016)
8	Benzo[a]pyrene	*Mycobacterium* sp. NJS-P	50	7.0	37	1.0	99%	Zeng et al. (2017)
9	Phenanthrene, Fluoranthene	*Klebsiella* sp., *Sphingomonas* sp.	20-100	7.2	30	30	74.32%, & 66.37% of PHN, FLT, respectively at 100 mg/L.	Xu et al., (2016)
10	Naphthalene	*Bacillus fusiformis*	25-150	7.3	30	2.0	99.8% at 50 mg/L	Lin et al. (2014)
11	Pyrene	*Pseudomonas* sp. Jpyr-1	10-200	7.0	28	7.0	100% at 200 mg/L	Ma et al. (2013)
12	Fluoranthene, Phenanthrene	*Pseudomonas sp. JM2*	50	6.0	37	4.0	40% each	Ma et al. (2012)
13	Phenanthrene, Fluoranthene, Pyrene	*Mycobacterium* sp. and *Sphingomonas* sp.	10	7.0	30	7.0	100% PHN, 71.2% FLT, 50% PYR	Zhong et al. (2011)
14	Benzo[a]pyrene	*Mycobacterium* sp NJS-1 and NJS-P	50-100	7.0	30	10	>80% each	Zeng et al. (2010)
15	Pyrene, Fluoranthene	*Burkholderia* sp. VUN10013	50	4.9	27	21	42.1 PYR, 41.1 FLT	Somtrakoon et al. (2008)

2.5. Summary of the literature and research gap

The demands for clean water are increasing day by day due to the rapid growth of population and industrialization. The wastewater discharged from industries such as textiles, papers, leathers, petroleum refineries, and petrochemicals are enriched with a wide range of pollutants and subsequently contaminate freshwater bodies like rivers, ponds, and lakes. Also, the wastewater containing pollutants such as dyes and aromatic hydrocarbons are toxic, carcinogenic, and mutagenic in nature and adversely affect the environment. These pollutants are the global concern amongst researchers. The physicochemical techniques such adsorption, filtration, ozonation, advanced oxidation, and photolysis, have been extensively used but these methods often suffer from high cost, phase transfer of the pollutants (e.g., adsorption), high sludge generation, toxic by-product generation. Therefore, extensive efforts are required to develop a cost-effective, environmentally benign, and energy-efficient technique to overcome the above anxiety.

In this direction, biodegradation is acknowledged as an eco-friendly and economical approach as compared to physicochemical methods. In biodegradation, the microorganisms can be used either free cells or immobilized cells. However, the immobilized cells are more promising in terms of high biomass of microbial population, high metabolic activity, and highly resistance to harmful pollutants, resulting high removal efficiency of pollutants. Although there are many research works going on in the field of biodegradation but slow degradation rate is a major challenge. For the application of immobilized cells, bioreactors play a key role in the biodegradation of dyes and aromatic hydrocarbons. However, very limited attention has been given to the continuous operation bioreactors (especially PBBR and MBBR) for the biodegradation of dyes and aromatic hydrocarbons. The application of acclimated and isolated bacterial species, continuous bioreactors, RSM, and growth-inhibition kinetics are the under-

explored area for the biodegradation of dye (Congo red) and aromatic hydrocarbons (naphthalene). The research gaps based on the literature survey are summarized in the following points given below.

1. Acclimatization and isolation of specific microbes are under-explored areas that have scope to enhance the rate of biodegradation.
2. Limited works are reported on the attached-growth bioreactor for the biodegradation of pollutants, especially on Naphthalene and Congo red dye.
3. Mass transfer limitation is a major concern in the attached-growth system due to limited mass transport.
4. Limited studies are available on continuous bioreactor for Naphthalene and Congo red degradation.
5. The application of RSM in the biodegradation process is an underexplored area, and it can enhance a greater yield with fewer experiments than the conventional one variable at time technique.
6. Few studies are available on the growth and inhibition kinetics of the above pollutants.