CHAPTER 2

LITERATURE REVIEW

CHAPTER 2

2.1. Biodegradation of phenol and its derivatives by fungi, yeast, and bacteria

2.1.1. Biodegradation by fungi and yeast

Fungi and yeast play a significant role in forming chlorinated phenols from the decomposition of organic matter. Various researchers have reported the mineralization ability of fungi for the utilization of phenol and its derivatives as carbon sources. Shebany et al. (2018) have isolated ten different fungi species (*Alternaria alternate*, *Aspergillus flavus*, *A. fumgatius*, *A. niger*, *A. terreus*, *Cladosporium cladosporioides*, *penicillium aurantiogriseum* etc.) and used for the biodegradation of phenol. White rot fungi, a group of microorganisms, showed significant removal efficiency of phenolic compounds (Tišma et al., 2010; Rubilar et al., 2008). Fouda et al. (2015) have evaluated the efficacy of *Aspergillus terreus* and *A. flavus* for the treatment of Bisphenol-A (BPA) and reported that the fungi could degrade BPA to the less toxic product. Similarly, various yeasts such as *Saccharomyces* sp., *Debaryomyces* sp., *Fusarium flocciferium*, *Trichosporon cutaneum*, *Candida* sp. were extensively studied for the biodegradation of phenol and its derivatives (Al-Khalid and El-Naas, 2012; Lakshmi and Sridevi, 2015). *Candida tropicalis* is more popular among researchers due to its high degradation ability (Basak et al., 2019; Subramaniam et al., 2020). Gong et al. (2021) have reported that *Candida tropicalis* isolated from the coastal soil could degrade 96 % of phenol at an initial concentration of 1800 mg/L. In addition, these microbial species are tolerant at high substrate and salt concentrations.

2.1.2. Biodegradation by bacteria

A large group of bacterial species (*Pseudomonas* sp., *Stenotrophomonas maltophilia*, *Bacillus* sp., *Alcaligenes*, *Acinetobacter*, *R. eutropha*) has been extensively studied for

biodegradation of phenol and its derivatives (Pradeep et al., 2015; Banerjee and Ghoshal, 2016). The significant resilient ability against adverse conditions of abiotic factors makes them more favorable for biodegradation purposes. Dey et al. (2019) have reported that *Bacillus cereus* removed 98.73 % of 4-chlorophenol at a concentration of 150 mg/L. Sandhibigraha et al. (2019) have investigated the effectiveness of *Bacillus subtilis* for 4-CP removal at an initial concentration of 1000 mg/L. Various researchers have analysed the efficacy of *Pseudomonas* and *Bacillus* species for the removal of phenol and its derivatives due to its phenol-tolerant nature (Saraireh et al., 2020; Sandhibigraha *et al*., 2019). Kumar et al. (2005) have reported that the *Pseudomonas putida* (MTCC 1194) could be able to utilize phenol (1000 mg/L) and catechol (500 mg/L) within 162 and 94 h, respectively. In aerobic conditions, phenol reduction undergoes two ways, such as ortho and meta cleavage pathways (Subramaniam et al., 2020). The proposed metabolic pathway during phenol biodegradation by bacteria is depicted in **Figure 2**. Initially, the phenol is oxidized in the presence of phenol hydroxylase to give the first intermediate compound, i.e., catechol (Singh et al., 2020). Catechol formation is the rate-limiting step in both ortho and meta cleavage pathways. It is a toxic intermediate product that is produced during the biodegradation of phenol. Then catechol is further oxidized by catechol 1, 2-dioxygenase to form cis, cis-muconate. Then cis, cis-muconate is successively degraded into 3-oxoadipate via lactonisation by muconate lactonizing enzyme. Finally, the 3-oxoadipate enters into the tricarboxylic acid (TCA) cycle to give the final product CO_2 and H_2O. In the meantime, catechol generated in the meta-cleavage pathway is degraded to 2-hydroxy muconic semialdehyde by catechol 2, 3-dioxygenase enzyme (Al-Khalid and El-Naas, 2012). The dehydrolysis of 2-hydroxy muconic semialdehyde to form 2-oxo-penta-4-enoate. Then, the product is reduced to form acetaldehyde and pyruvate, which enter into the TCA cycle. The summary of various

microorganisms, source of isolation, process conditions, and removal efficiency in biodegradation of phenol and its derivatives are given in Table 2.1.

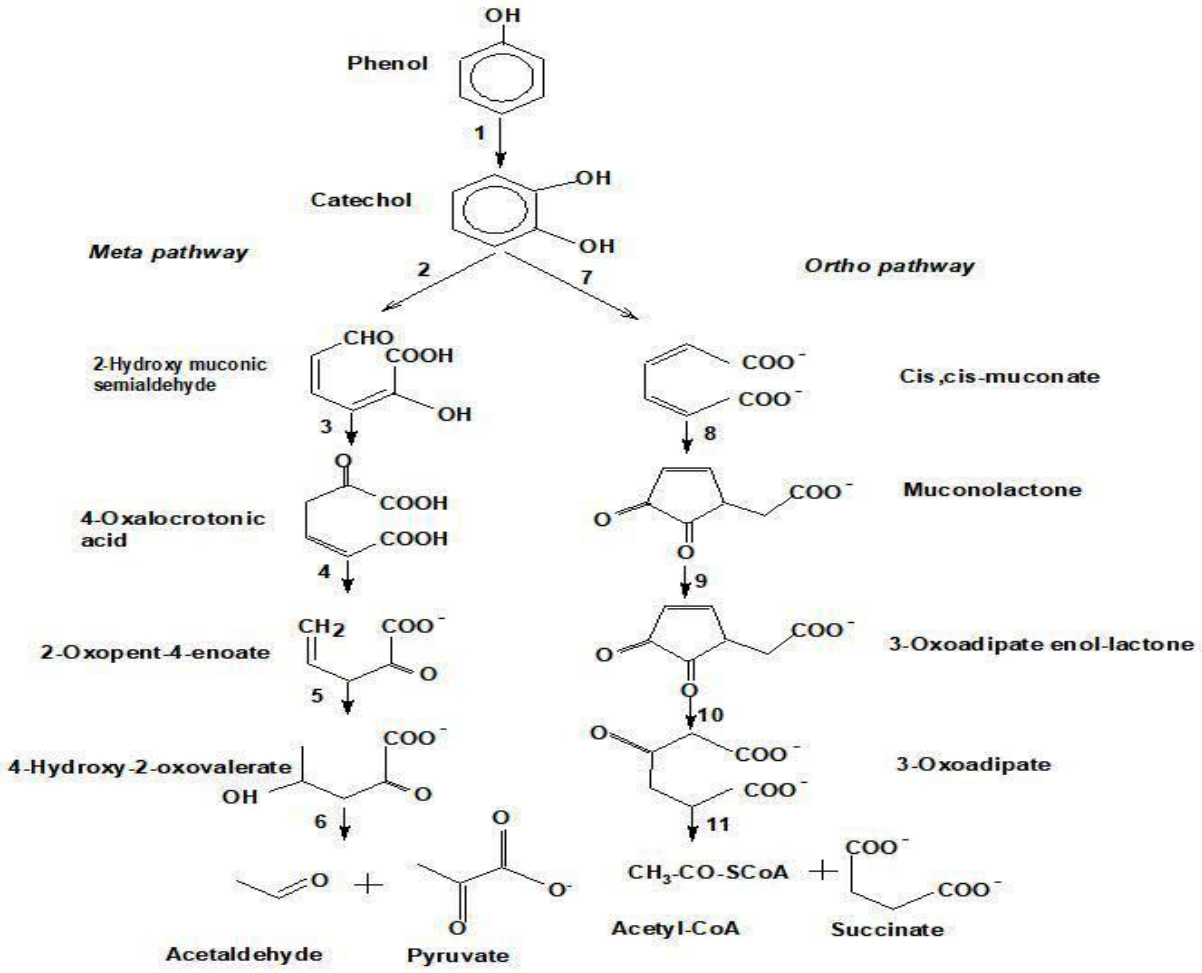

Figure 2. The metabolic pathway for phenol biodegradation in aerobic condition via meta and ortho pathways, (1) phenol monoxygenase, (2) catechol 2,3-dioxygenase, (3) Dehydrogenase, (4) Decarboxylase, (5) 2-keto-4-pentenoate hydratase, (6) Aldolase, (7) catechol 1,2-dioxygenase, (8) Lactonizing enzyme, (9) Isomerase, (10) oxoadipate enol-lactone hydrolase, (11) Transferase.

Table 2.1. A summary of microbial cells used for biodegradation of phenol and its derivatives.

S.N.	Phenolic compounds	Concentration (mg/L)	Microorganisms	Source of isolation	System (Free cell/Immobilized cell)	pH	Temperature (°C)	References
1	Phenol	50 – 1500	*Bacillus cereus*	Refinery site	Immobilized cell	7.0	30	Banerjee and Ghoshal, 2016
2	Phenol	400-2800	*Candida tropicalis*	Coke oven effluent	Immobilized cell	7.4	30	Basak et al., 2019
3	Phenol	150 – 1000	*Pseudomonas putida*	Sewage	Free cell	6.8-7.2	30-32	Mohanty and Jena, 2017
4	Phenol	200 – 1700	*Acinetobacter calcoaceticus*	Refinery effluent	Free cell	8.0	30	Liu et al., 2016
5	4-CP	1000	*Bacillus subtilis*	Automobile denting and painting service center	Free cell	7.4	37	Sandhibigraha et al., 2019
6	Phenol	0 – 1000	Ewingella americana	Wastewater treatment plant	Free cell	7.5	37	Khleifat, 2006
7	Phenol	200	*Ralstonia pickettii*	Refinery oil sludge	Free cell	8.5	35	Al-Zuhair and El-Naas, 2012
8	Phenol	1500	*Pseudomonas citronellolis*	Coke oven wastewtaer	Free cell	7.4	36	Panigrahy et al., 2020
9	4-CP	50 – 250	*Acinetobacter* sp. *Rhodococcus* sp.	Chemical industry wastewater	Free cell	7.5	28	Paisio et al., 2014
10	Phenol	1806	*Rhodococcus ruber* C1	Landfill	Free cell	7.0	20-45	Zhao et al., 2021
11	Phenol	750-1750	*Bacillus* brevis	Resin industrial wastewater	Free cell	8.0	34	Arutchelvan et al., 2006
12	Phenol	500 - 2500	*Corynebacterium* sp. DJ1	Sewage wastewater	Free cell	7.0-8.5	30	Ho et al., 2009
13	O-cresol	100-500	*Aspergillus fumigates*	Purchased	Free cell	6.6	31	Balamurugan and Preetha, 2014

14	4-CP	100 – 380	*Rhizobium* sp. 4-CP-20	Phenolic contaminated site	Free cell	6.89 - 8.20	36	Yang and Lee, 2008
15	Phenol	25 – 300	*Actinobacillus* sp.	ND	Free cell	7.0	35-37	Khleifat, 2007
16	2,4-DCP	100-300	*Bacillus sutilis*	Pulp and paper effluent	Free cell	8.0	37	Farag et al., 2021
17	4-CP	20	*Pseudomonas testosterone* *Pseudomonas solanacearum*	purchased from KAIST laboratory	Immobilized cell	7.2	30	Kim et al., 2002
18	P-NP	500	*Achromobacter denitrifacians*	Pharmaceutical effluent	Free cell	7.5	35	Mole et al., 2021
19	P-cresol	10-700	*Gliomastix indicus*	Pulp and paper effluent	Free cell	6.0	28	Singh et al., 2008

4-CP: 4-Chlorophenol; 2,4-DCP:2,4-Dichlorophenol, P-NP: Para-Nitrophenol.

2.2. Biodegradation of phenol and its derivatives

2.2.1. Free cell system

The phenomenon of the bacterial degradation of phenolic compounds draws significant attention from researchers. Bioremediation has been categorized into two methods for the practical application of the living cell: free cell and immobilized cell system (Basak et al., 2019).

Most of the free cell system is based on conventional activated sludge process. In this process, the bacterial cells remain in suspended form and degrade the organic matter present in the system to CO_2, H_2O, and biomass in the presence of oxygen. Air is supplied through a diffuser into the activated sludge tank to ensure the proper microbial growth. The sludge generated from the system is transferred to a secondary clarifier for disposal purposes. This process is a very popular technique due to low operating cost and operational easiness (Tay,

2006). However, the major drawbacks of this system are the inability to perform at high phenol concentration, entrainment/washout of the biomass, and poor settleability. Due to the toxicity effect, the direct exposure of the bacteria causes the death of the cell at a high pollutant concentration. This system can be unreliable and inefficient for the biodegradation of phenolic compounds above a loading rate of 1.0 kg/m^3.d (Tay et al., 2005).

2.2.2. Immobilized system

The inhibition of bacterial growth at high phenol concentration can be overcome by using the immobilization technique. The bacterial cells are immobilized onto the various packing supports to enhance the biodegradation by increasing the resistance against high concentrations of phenolic pollutants, adverse process conditions (pH, temperature, etc.), and complete washout of biomass from the system (Bharti et al., 2019; Banerjee et al., 2016). Various packing supports have been used for the immobilization of microorganisms such as natural polymers (sodium alginate, agar-agar, chitosan, etc.), synthetic polymers (polyvinyl alcohol, low-density polyethylene, polyurethane foam, high-density polyethylene, polypropylene), bio-wastes (sugarcane bagasse, luffa sponge, corncob, and sawdust), and activated carbon (Paisio et al., 2014; Banerjee et al., 2016; Basak et al., 2019). Jiang et al. (2013) have used *Acinetobacter* sp. immobilized on PVA (polyvinyl alcohol) for biodegradation of phenol and reported that the immobilized cell has comparatively higher phenol degradation and tolerant capability (against extreme conditions of pH and temperature) than free cells. However, substrate diffusion limitation is a major concern in this technique (Khalid and El-Naas, 2012; Basak et al., 2019).

2.3. Bioreactors

For the practical application of microbial cells, either in the free or immobilized system, various bioreactors were developed and studied for the biodegradation of toxic pollutants,

including phenol and 4-CP. The bioreactors are the most appropriate treatment technology in ex-situ biodegradation. The brief analysis on the use of different bioreactors for the biodegradation of phenol and its derivatives is represented in Table 2.2. The bioreactor selection is mainly based upon handling, operation, cost, and efficacy in removing pollutants. The bioreactor should also accommodate a high organic loading rate and active biomass generation. In this aspect, various bioreactors, such as sequencing batch reactors (SBRs), packed bed bioreactors (PBBRs), rotating biological contactors (RBCs), airlift bioreactors, and moving bed biofilm reactors (MBBRs), have been used for the biodegradation of 4-CP (Assadi et al., 2020; Azizi et al., 2021; Patel and Kumar, 2016; Wang et al., 2019).

2.3.1. Sequencing batch reactor

The sequencing batch reactor (SBR) is generally a kind of activated sludge process involving biodegradation in a single tank. The SBR is a batch process that consists of 5 cyclical periods such as fill, react, settle, decant, and idle. Each period can be adjusted to meet the desired concentration of the effluent. The major advantages of SBR over other activated sludge processes are small space requirement, ease of operation, simple design (Tay, 2006). Yussof et al. (2016) have reported that the inhibitory effect was observed in SBR at a high concentration of phenol, decreasing the removal efficiency. In addition, foaming, the poor settling ability of flocs, and size distribution are the primary concern of this process. The new activated sludge technique named granular activation sludge has been developed and extensively used for biodegradation of phenolic compounds to overcome this problem. In this technology, activated sludge of granular shape has been used in SBR. The size of the granules was in a range of 0.35 – 0.6 mm. Nevertheless, excellent floc settling velocity, a long time for granule formation, and inefficacy in low strength wastewater make the researchers find some effective biodegradation techniques (Alattabi et al., 2017).

2.3.2. Airlift bioreactor

The airlift bioreactor (ALBR) consists of a draft tube inside the bioreactor and an air diffuser situated inside or outside the tube. The density difference at the bottom and top of the reactor leads to fluid circulation and immobilized carriers from inside the reactor. Depending upon the location of the diffuser, it is classified into two categories: internal loop airlift bioreactor (ILALBR) and external loop airlift bioreactor (ELALBR). Less power consumption, simple construction, and operation are the major advantages of this technology. Loh and Liu (2001) have examined the efficacy of the ALBR for high-strength phenolic wastewater and observed that the ALBR could degrade phenol up to 300 mg/L by using *pseudomonas putida*. However, cellular damage due to the decoupling of gas contact with the small solid particle is a limitation found in this bioreactor. Complete biodegradation of the mixture of phenolic compounds (such as phenol, o-cresol, and p-nitrophenol) at an organic loading rate of 0.61 g COD L^{-1} d^{-1} was observed in a continuous ALBR by using *Acinetobacter* sp. and ammonia-oxidizing bacteria (Ramos et al., 2016).

2.3.3. Packed bed bioreactor

Packed bed bioreactor (PBBR), also known as a fixed-film bioreactor, uses immobilized solid packing material inside a cylindrical reactor for biodegradation. The objective is to obtain a high efficiency over a free cell system within a compact volume. The major benefits of PBBR include low cost, simple design, operation at a high organic loading rate, and high active biomass development (Kim et al., 2002, Basak et al., 2019). The fixed bio carrier plays a crucial role in biodegradation in PBBR as its physiological nature affects biomass density. The biocarriers should have various characteristics such as chemically inert, high porosity, reusability, and high surface area to volume ratio. Polyvinyl alcohol (PVA), calcium alginate,

low-density polyethylene, polyurethane foam, polypropylene, cocoa peat, luffa sponge, sand, gravel, sugarcane bagasse, corncob, etc., are the various packing materials used for the immobilization of microorganisms. The PBBR filled up with *Candida tropicalis* immobilized onto sugarcane bagasse could remove 97 % of phenol at a concentration of 2400 mg/L (Basak et al., 2019). Similarly, Sahoo and Panigrahy (2018) have evaluated the effectiveness of a PBBR packed with immobilized calcium alginate beads and found that complete biodegradation was obtained at an initial 4-CP concentration of 250 mg/L. Other researchers have successfully examined the efficacy of PBBR for the biodegradation of phenolic compounds (Banerjee et al., 2016; Jiang et al., 2007). However, mass transfer diffusion from bulk liquid to the bacterial site is the major drawback of this technology. In addition, the growth of excess biomass on the packing support creates a dead zone, and the interior bacteria undergo a starvation period (Sarti et al., 2001).

2.3.4. Moving bed biofilm reactor

The biodegradation of phenolic compounds in moving bed biofilm reactors (MBBRs) has been widely studied due to their excellent performance and unique characteristics. In MBBR, the air is provided at the bottom of the reactor to ensure the movement of the biocarriers inside the reactor. Due to the internal circulation of fluid, the substrate diffusion resistance between the bulk liquid to the microbial site reduces, and hence the better removal occurred. It has been reported that the high removal efficiency of the pollutant was obtained in MBBR compared to the stirred tank bioreactor (Gonzalez et al., 2001). Nakhli et al. (2014) have reported the effective removal of phenol during organic and hydraulic shock loading. Unlike PBBR, the fouling or clogging of the bed is minimum in MBBR; hence frequent cleaning is not required (Bassin and Dezotti, 2018). However, some demerits are still associated with MBBR, such as biomass detachment and energy cost due to aeration. The use of highly porous biocarriers could eliminate the biomass detachment problem. In this direction, various

biocarriers like polypropylene, polypropylene-polyurethane foam, polyethylene, and ceramic were developed and used in MBBR (Geed et al., 2017; Rahmat et al.,2016).

2.3.5. Rotating biological contactor

Rotating biological contactor (RBC) consists of a disc, rocks, or plastic media, which provide support for bacterial growth. It consists of horizontal plastic discs attached to a common shaft and partially submerged in wastewater. The major advantages of RBC include compatible design, easy construction, and no requirement of sludge recirculation. The energy cost is comparatively lower than activated sludge due to the less resistance offered by the sludge to the thin plastic disks. The rotation of the mechanical shaft results in the reduction of the retention time, and hence it decreases the duration of exposure to the toxicity of phenolic compounds. Various researchers have investigated the efficacy of RBC for phenolic compounds biodegradation under a high organic loading rate (Israni et al., 2002; Jeswain and Mukherjee, 2012). Sahinkaya and Dilek (2006) have reported the high removal efficiency of 4-CP at an organic loading rate of 1202 mg/L. d. They observed no significant change in performance when phenol concentration was increased from 200 to 822 mg/L, which implies the strong robustness of RBC against shock loading of 4-CP. Due to scale-up difficulty, time-consuming start-up, and oxygen diffusion resistance are the limitations in this technology (Hassard et al., 2015).

2.3.6. Trickling Biofilter

Trickling biofilters (TBFs) are a kind of static bed reactor filled up with different packing layers. The contaminated fluid is passed through the packing layers and degraded by the bacteria immobilized on the packing media. The TBFs are mostly used for gaseous volatile organic compounds (VOCs), including phenol and 4-Chlorophenol. Less hydraulic retention time and the high volume of fluid handling are the major advantages of this technology. The

effectiveness of a TBF filled up with *Pseudomonas pickettii* on poraver has been investigated for phenol biodegradation at a high OLR of 32.2 m^3 m^{-2} d^{-1} (Sa and Boaventura, 2001). Various researchers have studied TBFs for the biodegradation of phenolic compounds (Poi et al., 2017; Khalid and El-Naas, 2012). However, clogging of biofilter beds, the inconsistency of bioreactor at fluctuating concentrations, complex design, the requirement of skilled manpower are the limitations of TBFs (Kumar et al., 2011). The wood waste leachate (phenol, 4-Nitrphenol, 2-Chlorophenol, and o-Cresol) containing total phenol (1946 – 3810 µg/L) have completely treated in a TBF containing mixed consortium immobilized peat (Kamal et al., 2017). However, the color problem of the effluent needs further treatment.

Table 2.2. Various bioreactors used for biodegradation of phenol and its derivatives.

S.N.	Phenolic concentration (mg/L)	Bioreactor	Packing material	Microorganisms	Maximum removal efficiency (%)	References
1	3561	PBBR	Calcium alginate	*Bacillus* sp.	97.47	Banerjee and Ghoshal, 2017
2	2500	TPPB	Polymer tube	Mixed consortium	98	Angelucci et al., 2017
3	200	MBBR	Lignite activated coke	Activated sludge	94.75	Zheng et al., 2019
4	408	MBBR	Polyethylene	Bacterial consortium	84.8	Li et al., 2011
5	13	RBC	_	*Bacillus* sp. *Pseudomonas* sp.	99	Khondabi et al., 2019
5	200	Stirred tank bioreactor	_	*Rhodococcus* Sp.	100	Paisio et al., 2012
6	25	SBR	_	*Bacillus* sp.	88	Sonkar et al., 2019

7	2545	FBBR	Calcium alginate	*Bacillus cereus*	98.03	Banerjee and Ghoshal, 2016
8	4300	UASB	_	Activated sludge	81	Gonçalves et al., 2012
9	60	MBBR	Polyethylene	Activated sludge	86	Brink et al., 2017
10	2400	PBBR	Sugarcane bagasse	*Candida tropicalis*	97	Basak et al., 2019
11	250	ALR	_	Mixed consortium	99	Patel and Kumar, 2016
12	200	SBR	_	Activated sludge	100	Sahinkaya and Dilek, 2006
13	500	EIFBAB	Polystyrene beads	*Pseudomonas putida*	100	Loh and Liu, 2011
14	250	PBBR	Calcium alginate	*Arthrobacter chlorophenolicus*	98.6	Sahoo and Panigrahy, 2018
15	800	MBBR	HDPE	Mixed consortium	99	Nakhli et al., 2014
16	100	ALPBB	Plastic media	Activated sludge	40	Azizi et al., 2021

PBBR: Packed bed bioreactor; TPPB: Two-phase partitioning bioreactor; ALBR: Airlift bioreactor; FBR: Fluidized bed bioreactor; SBR: Sequential batch reactor; MBBR: Moving bed biofilm reactor; RBC: Rotating biological contactor; Up-flow anaerobic sludge blanket reactor; EIFBAB: External loop inversed fluidized bed airlift bioreactor; HDPE: High density polyethylene; ALPBB: Airlift packed bed bioreactor.

2.4. Findings of the literature review and research gap

The high demand for potable water promotes the treatment of wastewater discharged from various industries, agriculture, and other human activities. The pollutants such as polycyclic aromatic compounds (PAHs), BTEX (benzene, toluene, and ethylbenzene isomers), phenol, heavy metals, pesticides, and dyes are generally found in the industrial wastewater. Most of these compounds are recalcitrant, carcinogenic, and toxic to human

beings and ecosystems. Phenol and its derivatives are versatile intermediates used in various industries like resin, oil refinery, textile, pharmaceutical, and leather. These compounds can cause respiratory dysfunction, renal toxicity, and growth inhibition in human and aquatic lives. It is also lethal to fish and algae at low concentrations. Various conventional methods such as advanced oxidation processes (e.g., Fenton, ozonation), membrane separation, electrolysis, and biodegradation have been applied for the treatment of phenol and its derivatives. Among these methods, the biodegradation is preferred primarily due to its cost-effectiveness and eco-friendly nature. Traditionally, bioremediation is carried out via two processes: *free/suspension cell* and *immobilized cell system*. The free cell systems offer a low biodegradation rate, particularly at a high concentration of pollutants. However, the immobilized biomass cell onto packing supports offers several merits, including large surface area, high biomass growth, and withstand under adverse environmental conditions. The bioreactors, namely PBBRs and MBBRs, have been extensively used for the biodegradation of various organic pollutants. However, biodegradation of phenol and its derivatives in the attached growth bioreactors, effect of mass diffusion, biogenic substrate, substrate utilization rate, and growth kinetics were less researched topics reported in the literature. Based on the literature review, the following research gaps are found as:

1. Isolation and acclimatization of potential microorganisms obtained from the contaminated site are under-explored areas and have the scope to enhance the rate of biodegradation
2. External mass transfer plays a major role in the performance of bioreactor during the biodegradation and has been seldom included in the bioreactor studies
3. Limited studies are reported on the continuous operation of the bioreactors (packed bed bioreactor and moving bed biofilm reactor) for the biodegradation of phenol and its derivatives

4. Few literatures are found on the effect of biogenic substrates on the biodegradation of phenol and its derivatives
5. No study is available on the comparative analysis between various attached-growth bioreactors, i.e., packed bed bioreactor (PBBR) and moving bed biofilm reactor (MBBR) for biodegradation of phenol and its derivatives

2.5. Objective of the work

The present work was focused on investigating the efficacy of biodegradation of phenol and its derivatives (such as 4-chlorophenol) in the PBBR and MBBR using potential bacterial species isolated from the samples obtained from an oil contaminated site. The specific investigations performed in this study are laid out as following:

1. Isolation, and identification of potential microorganisms obtained from an oil contaminated site followed by the process parameters optimization for maximum removal of phenol and its derivatives in a free cell system
2. Biodegradation of phenol in a PBBR and the effect of external mass transfer barrier on biodegradation
3. Performance evaluation of the MBBR for biodegradation of phenol and 4-chlorophenol
4. A comparative analysis between the PBBR and the MBBR operated under identical conditions for the biodegradation of 4-chlorophenol

The methodology and results section of the thesis have been divided into three subsections for the convenience as follows:

Section A: Experimental works relating to biodegradation of phenol

Section B: Experimental works relating to biodegradation of 4-chlorophenol

Section C: Experimental works relating to comparative analysis between the packed bed and moving bed bioreactors