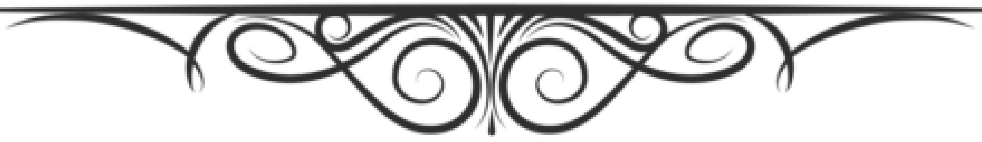
Chapter 2: Literature Review



Among the various methods for the removal of harmful pollutants, biodegradation stands out as the most favorable and widely accepted due to its environmentally friendly, cost-effective, and realistic feasibility (Zhou and Nemati, 2018). Bioremediation has emerged as a viable technique for removing phenolic pollutants into harmless end products like CO_2 and H_2O (Wu et al., 2022). Microorganisms are utilized pollutants as carbon sources (Sun et al., 2019). Microbial species responsible for the biodegradation of phenolic pollutants are described below:

2.1. Biodegradation of phenolic compounds through algae

Algae are a kind of microorganisms that eliminate pollutants and capture carbon. Complete biodegradation through microalgae of phenolic compounds follows two primary phases: (a) the division of any phenolic ring substituent and (b) the cleavage of the phenolic ring. Recently, there has been a growing focus on algal technology for breaking down phenolic compounds, particularly in the treatment of real-world industrial wastewater (Nur et al., 2021; Stephen and Ayalur, 2017). Based on the differences in the structure of petroleum hydrocarbons, the biodegradability simplicity follows the order of mono aromatics > cycloalkanes > polyaromatics (Li and Meng, 2023). Among various algae, *Scenedesmus* and *Chlorella* are very popular and widely used in the biodegradation of different pollutants (Surkatti and Al-Zuhair, 2018). Xiao et al. (2019) used *Chlorella vulgaris* for the biodegradation of wastewater from coal gasification plants containing *p*-cresol (400 mg L^{-1}) and phenol (800 mg L^{-1}). They found lower concentrations (100 mg L^{-1}) of phenol notably enhanced the degradation of *p*-cresol during co-metabolism. Meza-Escalante et al. (2022) used *Tetraselmis suecica*, type of green algae, to degrade *p*-cresol (20 mg L^{-1}) under pH of 7.0 and a temperature of 20 °C. They achieved almost complete removal efficiency within 72 hours. Wei et al. (2020) utilized the green algae *Chlamydomonas reinhardtii* to degrade nitrophenol (15 mg L^{-1}) under pH ranging from 7.0 to 8.5 and a temperature of 26 °C. They observed complete removal efficiency (100%) within 6 hours.

2.2. Biodegradation of phenolic compounds through bacteria

Out of the various microorganisms, bacteria emerge as the most promising contenders for the biodegradation of phenolic compounds in polluted wastewater. Consequently, extracting bacteria capable of degrading these compounds from contaminated environments is a highly prevalent and widely embraced approach (Panigrahy et al., 2022b). Numerous bacterial strains (*Pseudomonas*, *Sphingomonas*, *Ralstonia*, *Cupriavidus*, *Burkholderia*, *Acinetobacter*,

Rhodococcus, and *Arthrobacter,* etc.) are capable of metabolizing phenols and their derivatives. These strains were thoroughly examined biochemically and genetically to understand their characteristics (Rucká et al., 2017). Mohanty and Jena (2017) used the bacterial species *Pseudomonas putida* NBM11 to efficiently degrade phenol (1000 mg L^{-1}) within a pH range of 6.8 to 7.2 at temperatures between 30 and 32 ºC, achieving a removal efficiency of 95%. Xenofontos et al. (2016) they used *Advenella sp.* LVX-4 to biodegrade *p*-cresol (750 mg L^{-1}), achieving removal efficiency of 100% after 432 hours at a pH of 8 and temperature of 30 ºC. Yang et al. (2018a) utilized a bacterial mix comprising *Aspergillus sp.* NPF2 and *Aspergillus sp.* NPF3 for the degradation of 4-n-nitrophenol (20 mg L^{-1}). They achieved 86.0% and 98.8% removals using *Aspergillus sp.* NPF2 and *Aspergillus sp.* NPF3, respectively, within 72 hours. Enhancing the removal of phenolic pollutants can be achieved by employing a nitrifying consortium. This usually involves using ammonia oxygenase from nitrifiers through co-metabolism, which not only enhances the tolerance of nitrifiers to toxicity but also speeds up the biodegradation of chlorophenol (Panigrahy et al., 2022a).

Li et al. (2020) studied the simultaneous removal of 4-Nitrophenol (4-NP) and NH_4-N using nitrifying bacterial consortium *Ignavibacteria* and *Aeromonas* in sludge bioreactor and they documented 99.9% removal of 4-NP and 99.5% removal of NH_4-N. They also studied the stability of nitrifying sludge in two sequencing batch reactors (SBR) fed with 60 mg L^{-1} of 2-CP (2-chlorophenol) and varying starting ammonium concentrations in the 100–500 mg L^{-1} range. Regardless of the rise in ammonium concentration or the operational cycles, the sludge completed nitrification in 14 days, resulting in ammonium consumption efficiencies of nearly 99% and nitrate generation yields of 0.93 to 0.99. 2-CP almost completely degraded within 7.0 days. The result reveals that the nitrifying system is an appropriate strategy to increase the co-metabolic degradation of recalcitrant pollutants. Sometimes, it looks more promising to utilize a mixture of bacteria to degrade phenolic compounds since it has several advantages. Some of these include a significant toxicity tolerance, and sometimes bacterial consortiums secrete functional enzymes that quickly break down pollutants without producing harmful substances (Elumalai et al., 2021). On the other hand, depending on specific bacterial strains may result in the build-up of hazardous by-products because of their restricted capacity to generate essential enzymes (Patel and Kumar, 2016). Zielińska et al. (2014) used microbial consortium to remove bisphenol A and found 92% removal efficiency in 1.5 hours (HRT, (hydraulic retention time)) was achieved.

2.3. Biodegradation of phenolic compounds through fungi

Fungi are prominent tools for the degradation of recalcitrant pollutants such as phenols, naphthols, bisphenols, phenolic or non-phenolic textile dyes, halogenated aromatics, polyaromatic hydrocarbons, etc. (Martínková et al., 2016). Compared with bacteria, fungi, especially yeast, have unique peroxisomes that play a prominent role in forming reactive oxygen species (Lismont et al., 2015). For the treatment of phenolics-contaminated wastewater, *Candida tropicalis sp*. (He et al., 2022), *Basidiomycota* (Martínková et al., 2016), *Debaryomyces sp*. (Jiang et al., 2017), *Thielavia sp*. HJ22 (Mtibaà et al., 2020), etc., have been used. Various fungal species, particularly basidiomycetes, have emerged as highly suitable options to remove wide range of toxic aromatic pollutants. This is mainly due to their release of external peroxidases or the existence of internal enzymes such as *Cytochrome* P450 monooxygenases (Mtibaà et al., 2020).

In a study, Jiang et al. (2017) used *Debaryomyces* species immobilized in calcium alginate beads with nanoscale Fe_3O_4 and found remarkable efficiency in the degradation of phenol. They achieved 99.9% degradation at 900 mg L^{-1} phenol within 80 hours. The immobilized *Debaryomyces* species retained their efficacy and could be reused for up to 10 cycles. The *Basidiomycota*, renowned for their secretion of phenolic-degrading enzymes compared to others such as *laccases, tyrosinases*, *manganese*, and *lignin peroxides*, demonstrated remarkable capability to remove phenolic compounds during biodegradation processes (Panigrahy et al., 2022a).

2.4. Biodegradation of phenolic compounds through laccase

Laccase, predominantly generated by fungi like white-rot varieties and certain bacterial strains, serves as a polycopper oxidase. It acts as a green catalyst possessing distinct inherent characteristics, facilitating substrate oxidation through single-electron processes in the presence of molecular oxygen (Chen et al., 2022). Laccase from various origins displays varying redox potentials, whereas fungal laccases notably demonstrate a higher redox potential ranging between 470 to 810 millivolts (Chen et al., 2022). Due to its exceptional catalytic properties, laccase has been extensively and effectively applied in wastewater treatment and bioremediation industries to successfully degrade phenolic compounds (Mukherjee et al., 2013). Moreover, the limitations of free laccases, such as their susceptibility to harsh industrial environments and inadequate durability, need improvement. Enzyme immobilization is an important and highly sought-after technology in wastewater

treatment. It's a well-established approach for reusing effective catalysts, separating products, and facilitating their recycling (Bilal et al., 2020).

Rodríguez-Delgado et al. (2016) investigated the biotransformation of micropollutants such as diclofenac sodium (DFC), 5,7-Diiodo-8-hydroxyquinoline (DHQ), β-Naphthol (β-NP), and 2,4-dichlorophenol (2,4-DCP) utilizing a laccase cocktail (LacI/LacII) derived from *P. Sanguineus* CS43 in both synthetic buffers and real groundwater samples. In synthetic samples, 50% degradation was attained for DCF, 78% for DHQ (), 97% for β-NP, and 2,4 71% for using the laccase enzyme sans mediators.

Qiu et al. (2020) immobilized laccase on a biocarrier prepared by using the Schiff base process, in which dialdehyde starch and Fe_3O_4 magnetic nanoparticles modified with amino-functionalized ionic liquid were mixed, known as Fe_3O_4-NIL-DAS. The immobilized laccase enhanced efficacy in removing phenolic compounds (such as phenol, 4-chlorophenol, and 2,4-dichlorophenol) across various temperatures and pH levels. This exhibited maximum removal efficiencies of 86.1%, 93.6%, and 100% for phenol, 4-chlorophenol, and 2, 4-dichlorophenol, respectively. The laccase also demonstrated remarkable stability, retaining 83.5% of its initial activity after six cycles.

2.5. Review on biodegradation of phenolic compounds

Swain et al. (2022) optimize process variables such as mixing intensity (60–140 rpm), phenol concentration (50–200 mg L^{-1}), and HRT (4–24 h) in a moving bed biofilm reactor (MBBR), using *Bacillus cereus* GS2 IIT (BHU) immobilized on polyethylene biocarriers. The optimum conditions for phenol removal (87.64%) were identified at 100 rpm mixing intensity, 200 mg L^{-1} phenol concentration, and 24-hour HRT. Increasing mixing intensity notably enhanced substrate diffusion between the liquid phase and biofilm surface. The study determined external mass transfer coefficients between 1.431×10^{-5} to 1.845×10^{-5} m s^{-1}. Additionally, catechol and 2-hydroxymuconic semi-aldehyde detection indicated that *Bacillus sp*. followed the meta-cleavage pathway during phenol biodegradation.

Singh et al. (2022) studied the kinetics analysis of eliminating *p*-cresol from wastewater using the bacterial strain *Serratia marcescens* ABHI001 in batch shake flasks. They analyzed the effect of concentrations of *p*-cresol (50 to 500 mg L^{-1}), inoculum dosage (2%, 5%, 8%, 10%, 12%, and 15%), temperature (25, 30, 30, 35, and 40 °C), pH (3, 4, 5, 6, 7, 8, and 9) and agitation (80 to 180 rpm). Results showed that a 10% v/v inoculum of 24 hours effectively degraded *p*-cresol. The concentrations above 100 mg L^{-1} had an inhibitory effect. The maximum specific growth rate μ_{max} was 0.360 h^{-1} at a 100 mg L^{-1} concentration. The study

successfully fitted experimental data to Haldane's and Andrew's models. For Haldane's model, μ_{max}, K_S, and K_I were 0.9697 h^{-1}, 88.07 mg L^{-1}, and 219.9 mg L^{-1}, respectively. For Andrew's model, μ_{max} was 0.6917 h^{-1}, K_S was 62.83 mg L^{-1}, and K_I was 307.4 mg L^{-1}. Additionally, the yield coefficient for growth on *p*-cresol was found to be 0.82.

Patel et al. (2022) studied the biodegradation of 4-chlorophenol (4-CP) using *Bacillus subtilis* (MF447841.1). Both batch and column experiments were conducted to optimize parameters (initial concentration, contact time, and inoculum dosages) for efficient 4-CP biodegradation. Levenspiel's model, with an R^2 value of 0.97, was the best fit among other models. The specific growth rate (μ), yield of cell mass ($Y_{\frac{x}{s}}$), and saturation constant $K_S$were determined as 0.63 (h^{-1}), 0.35 (g/g), and 0.006884 (g L^{-1}), respectively. The isolated strain exhibited the ability to degrade 4-CP up to 1000 mg L^{-1} initial concentration within 40 hours. Further, experiments involved immobilizing the bacterial strain in calcium alginate beads. A packed bed reactor (PBR) study optimized various process parameters such as initial feed concentration, bed height, and flow rate. The maximum biodegradation efficiency of 4-CP reached 45.39% at an initial concentration of 500 mg L^{-1} within 105 minutes. 2 mm size immobilized beads created with 3.5% w/v of calcium chloride and sodium alginate were used. The findings suggest that *Bacillus subtilis* (MF447841.1) holds promise for the biological remediation of 4-CP pollutants in wastewater. Additionally, due to its cost-effectiveness and environmentally friendly approach to water treatment, there's potential for its commercialization.

Basheer and Farooqi (2012) investigated the development of aerobic granules in a sequencing batch reactor to degrade the *p*-cresol. These stable granules (1 to 5 mm in diameter) exhibited an integrity coefficient of 96% and a density of 1046 kg m^{-3}. Their granules settling velocity ranged from 2×10^{-2} to 6×10^{-2} m sec^{-1}. These aerobic granules effectively decomposed *p*-cresol, achieving 88% removal efficiency at up to 800 mg L^{-1} concentrations. The specific degradation rate of *p*-cresol in the aerobic granules followed the Haldane model, indicating substrate inhibition. Notably, a high specific degradation rate of up to 0.96 g *p*-cresol (g VSS day)$^{-1}$ was sustained even at *p*-cresol concentrations of 400 mg L^{-1}.

Bera et al. (2019a) isolated *Stenotrophomonas sp*. DBK3 (MF004205) from a petroleum refinery. *Stenotrophomonas sp*. DBK3 exhibited resilience towards *p*-cresol, enduring a concentration of 600 mg L^{-1}. It completely degraded 400 mg L^{-1} of *p*-cresol completely within 189 hours. At a higher concentration of 600 mg L^{-1}, approximately 74% degradation efficiency was found. Various growth kinetics models were applied to ascertain bio-kinetics

Table 2.1 Summary of biodegradation of phenolic compounds using microorganisms

S.N.	Microorganisms	Phenolic compounds	Operating conditions	Reactors	% Removal	References
1	*Pseudomonas sp.*	*p*-Cresol	Initial conc. = 100 mg L^{-1}, pH 8, Temp = 30 °C,	Batch reactor	100% Time 32.7 h	Hamitouche et al., (2014)
2	*A. Calcoacticus, P. pseudoalcaligenes*	Phenol	Initial conc. = 94 mg L^{-1}	Free cell	>99% Time 10-20 h	Wei et al., (2016)
3	*Pseudomonas sp.*	*o*-Cresol	Initial conc. = 100 mg L^{-1}, pH 8, Temp = 30 °C, stirring velocity = 200 rpm	Batch reactor	99.9% Time 24 h	Hamitouche et al., (2015)
4	*Ochrobactrum sp.* SAR1, & SAR 3 *Pseudomonas sp.* SAR2 & consortium	Phenol	Initial conc. = 500 mg L^{-1}, pH 7, Temp = 37 °C,	Free cell	100% Time 96 h	Chandrasekaran et al., (2018)
5	*Advenella sp. LVX-4*	*p*-cresol	Initial conc. = 500 mg L^{-1}, pH 9, Temp = 30 °C,	Free cell	100% Time 432 h	Xenofontos et al., (2016)
6	*Bacillus sp.* SAS19 and *Corynebacterium sp.* SAS21 = 3:2 (Bacterial consortium)	Phenol	Initial conc. = 100 mg L^{-1}, pH 7, Temp = 30 °C,	Free cell	100% Time 8 h	Li et al., (2018)
7	*Kocuria sp.* IBETAN4 25 7–9	Phenol	Initial conc. = 471 mg L^{-1}, pH 7-9, Temp = 25 °C,	Free cell	100% Time 72 h	Wu et al., (2018)
8	*Aspergillus sp.* NPF2 and *Aspergillus sp.* NPF3	4-n-nonylphenol	Initial conc. = 26 mg L^{-1}	Free cell	86.0 % & 98.8 % Time 72 h	Yang et al., (2018)
9	*Chlorella pyrenoidosa*	Phenol	Initial conc. = 200 mg L^{-1}, pH 8, Temp = 35 °C,	Free cell	97.6% Time 168 h	Dayana Priyadharshini and Bakthavatsalam, (2019)
10	*Phanerochaete chrysosporium*	2,4-Dichlorophenol	Initial conc. = 20 mg L^{-1},	Free cell	100% Time 36 h	Huang et al., (2017)
11	Microbial community	Phenol	Initial conc. = 120 to 1200 mg L^{-1}	Anaerobic Sequencing	100% Time 50 - 220 h	Rosenkranz et al., (2013)

12	Yeast, mould and bacteria	Phenol	Initial conc. = 200-1200 mg L^{-1}, hydraulic retention time = 8–24 h, inlet salt content = 10–70 g L^{-1}	Batch Reactor Moving bed biofilm reactor	99%, HRT of 18 h	Nakhli et al., (2014)
13	*Rhizobium*, *Pseudomonas*	Phenol	Initial conc. = 1500 mg L^{-1}, Temp = 28 ± 3 °C, , pH 7.5 ± 0.1 and DO = 3–6 mg L^{-1},	Membrane bioreactor	100% Time 4 days	Su et al., (2019)
14	Arthrobacter chlorophenolicus A6	p-nitrophenol	Initial conc. = 600 and 1400 mg L^{-1}, HRT between 18 and 7.5 h	Up-flow packed bed reactor	99.9% HRT 18 h	Sahoo et al., (2011)
15	Indigenous mixed microbial culture	Phenol and Resorcinol	Initial conc. = 400 mg L^{-1} each	Aerobic batch reactor	100% each Time 58 h	Dey and Mukherjee, (2013)
16	*Gliomastix indicus* MTCC 3869	*p*-cresol	Initial conc. = 10- 700 mg L^{-1}, pH 6, Temp = 28 °C,	Free cell	<90% Time 308 h	Singh et al., (2008)
17	Stenotrophomonas *sp*.	*p*-cresol	Initial conc. = 400 mg L^{-1}, pH 7, Temp = 37 °C,	Free cell	100% Time 189 h	Bera et al., (2019b)
18	*Bacillus sp*. PHN 1	*p*-cresol	Initial conc. = 200 mg L^{-1},	Free cell	100 % Time 144 h	Tallur et al., (2009)
19	*Bacillus aryabhattai, Bacillus megaterium,* and *Bacillus cereus*	*p*-cresol	Initial conc. = 1000 mg L^{-1}	Denitrification baffled reactor	100 % Time 424 days	Mahdavianpour et al., (2018)
20	Mixed Culture	*p*-cresol	Initial conc. = 100 mg L^{-1}	Batch Reactor	100% Time 48 h	Hamitouche et al., (2012)
21	*Pseudomonas and Rhodococcus*	Phenol, *p*–nitrophenol and hydroquinone	Initial conc. = 247, 252, 253 mg L^{-1}	Membrane–aerated biofilm reactor	> 95% Time 180 day	Tian et al., (2020)
22	*Pseudomonas- putida*	Phenol	Initial conc. = 137 mg L^{-1}	Packed bed reactor	93.3 % and 95.9% were obtained at various HRTs between 3.1 and 10.5 h	Lin and Gu, (2023)

23	*Achromobacter sp.*	2,4-dichlorophenol	Initial conc. = 6.86 to 102.38 mg L^{-1}, pH 7.2	Air-lift honeycomb-like ceramic reactor	100 – 88 %	Xiangchun et al., (2003)
24	Activated sludge	p-nitrophenol	Initial conc. = 200 ± 50 mg L^{-1}	Membrane aerated biofilm reactor	94.40% (COD) 79.57% loads were 3.644 g m^{-1} d^{-1} and 4.975 g m^{-1} d^{-1}	Tong et al., (2022)
25	*Proteobacteria* and *Ascomycota*	*p*-nitrophenol	Initial conc. = 1250 mg L^{-1}	Aerated biological fluidized bed	COD and PNP removal 95% and 99% Time 45 day	Ji et al., (2017)
26	Activated sludge	p-nitrophenol	Initial conc. = 12.8 to 128 mg L^{-1}	Slurry bubble column	100 % HRT 2.5	Salehi et al., (2011)
27	*C. tropicalis*	Phenol	Initial conc. = 2400 mg L^{-1}, pH 6	Packed-bed column reactor	97% at 54 h^{-1}	Basak et al., (2019)
28	*Pseudomonas putida* (ATCC 17484)	Phenol	Initial conc. = 500 mg L^{-1}, pH 7.0	Packed-bed column reactor	93% at rate of 78.9 mg L^{-1} h^{-1}	Zhou and Nemati, (2018)
29	*Microbacteriun arborescens* and *Bacillus cereus*	Phenol	Initial conc. = 977 mg L^{-1}	Airlift-packed bed reactor	100%	Huang et al., (2010)
30	Co-culture of *Bacillus sp.* and *E. coli*	Phenol	Initial conc. = 100 mg L^{-1}	Packed bed bioreactor	90.9% Time 4 days	Gupta and Balomajumder, (2016)

parameter values. Haldane's model exhibited the most accurate fit with correlation coefficient value of 0.97. The associated kinetic parameters, μ_{max} = 0.1160, K_S = 43.53 mg L^{-1}, and K_I = 387 mg L^{-1} were obtained using Haldane model.

2.6. Summary of literature survey and research gap

Due to ongoing industrial demands for chemical raw materials and by-products produced during different activities, considerable amounts of effluent-containing harmful compounds are continuously discharged into aquatic and open atmospheres. Among various pollutants, phenolic compounds, the most toxic pollutants found in wastewater, originate from diverse industries such as petroleum refineries, plastic, paper and pulp, pharmaceuticals, and coal processing. They substantially affect living organisms and the environment through cytotoxic effects, even at minimal concentrations, producing highly toxic electrophilic by-products facilitated by the cytochrome P450 enzyme system. These potent by-products have the potential to bind to and harm the DNA or essential enzyme systems of living organisms, leading to mutagenic and carcinogenic effects. Hence, US and European Environmental Protection Agencies have designated these phenolic compounds as priority pollutants.

In recent decades, various traditional methods have been investigated in addressing phenolic wastewater treatment, including adsorption, Fenton reagent, ozonation, membrane-based technologies, electrochemical processes, and photocatalysis. Among these techniques for removing phenolic contaminants, biodegradation is the most favourable and widely accepted due to its multiple advantages, such as its eco-friendly characteristics, economic viability, and practical feasibility, making it a highly promising approach. Biodegradation occurs by directly employing or immobilizing bacteria within a suitable bio-carrier in the contaminated environment. Furthermore, the drawbacks of utilizing free bacteria for treating wastewater under harsh conditions encompass issues like substrate inhibition, susceptibility to environmental variations, and challenges associated with recovering and reusing the bacteria. The recent development of immobilizing microorganisms using suitable biocarrier materials aims to address these challenges. These techniques provide numerous advantages, such as protecting bacterial cells from severe environmental stresses, avoiding cell loss during continuous mode operation, lowering expenses through recycling, and attaining higher bacterial cell concentrations, resulting in enhanced degradation capabilities. Cell immobilization is typically accomplished through two

distinct approaches: cell entrapment and attachment. In the initial method, microorganisms are confined and physically confined within fibrous or porous materials. Various polymers, including alginate, chitosan, and cellulose derivatives, have served as matrices for entrapping cells. Conversely, within the cell-attachment procedure, microorganisms bind themselves to appropriate material surfaces through self-adhesion. Materials frequently employed in the cell-attachment approach consist of polyurethane foam (PUF), nylon sponge, and synthetic foams.

Both types of support materials are appropriate for microbial treatment of industrial wastewater. However, due to its outstanding porosity, adsorption ability, and strong mechanical strength, polyurethane foam (PUF) has been shown to be the most effective medium for ensnaring microorganisms. In bioremediation, bioreactors like sequencing batch reactors, packed bed bioreactors (PBR), airlift bioreactors, rotating biological contactors and moving bed biofilm reactors have been employed to degrade phenolic compounds. The effectiveness of these bioreactors relies on factors such as the type of bed used, characteristics of the packing material (like porosity, specific surface area, and compatibility with living organisms), the rate at which the compounds are introduced, resistance to transferring mass between the liquid and biofilm, and the availability of oxygen. PBR is the most practical reactor for effectively removing phenolic contaminants. The immobilized cells are used in PBR, which has several advantages, such as high effectiveness, easy scaling, automation potential, and the capacity to continuously treat large volumes of wastewater with a fixed number of immobilized cells.

The literature review highlights the gaps in research through the following summarized points:

- Isolation and acclimatization of potential microorganisms are under-explored areas that have the scope to enhance the rate of biodegradation.
- Lack of detailed exploration concerning *p*-cresol biodegradation in continuous mode operation.
- Lack of research addressing enhancing bacterial activity under various loading rate.
- Comprehensive research on the biodegradation of high *p*-cresol concentrations is not readily available.
- Simultaneous removal of phenolic and dye from wastewater.

2.7. Objective of research work

- Isolation and identification of potential bacterial species from activated sludge for the biodegradation of *p*-cresol
- Optimized various parameters for maximum *p*-cresol biodegradation.
- Performance evaluation of packed bed biofilm reactor on various loading rates.
- Integrating photocatalytic and biodegradation process to enhanced biodegradation of high concentration of *p*-cresol.
- Bacterial toxicity assessment of biodegraded product.
- Evaluation of phytotoxicity, chlorophyll content in *Vigna radiata* seeds and leaves, respective.