CHAPTER 1

INTRODUCTION

CHAPTER 1

1.1. Introduction

Massive industrialization and urbanization generate various pollutants that contaminate the water, soil, and air. The removal of harmful compounds from the wastewater has been essential due to the increasing scarcity of potable water worldwide. The organic and inorganic contaminants such as BTEX (benzene, toluene, ethylbenzene, and xylene isomers), phenol and its derivatives, polycyclic aromatic hydrocarbons (PAHs), pesticides, dyes, and heavy metals lead a remarkable role in water pollution (Bharti et al., 2019; Sonwani et al., 2019). These pollutants are mostly discharged in the water bodies from oil refineries, automobiles, polymeric resin production, pharmaceutical, pesticides, textile, and steel industries (Geed et al., 2017; Das et al., 2019; Mallick and Chakraborty, 2019). Phenol and its derivatives are extensively used as raw materials in various manufacturing industries such as resin manufacturing, drugs, azo dyes, fertilizer, metal coating, etc. These are classified as hydroxyl aromatic hydrocarbons like benzene ring structures containing phenyl or aryl groups. The high solubility and diffusivity of these compounds impact adverse effects on the ecosystems. The chemical structures of phenol and its derivative compounds are provided in Figure 1.

1.1.1. Sources and pollution

The sources of the phenol and its derivatives are either natural or anthropogenic. These are naturally produced from the decomposition and biosynthesis of plant materials. Under the exposure of Ultraviolet rays, these are produced from the amino acids present in the hemicelluloses. In addition, the breakdown of tyrosine in the animal intestinal tract produces phenol and p-cresol (Michałowicz and Duda, 2007). However, human activity plays a major role in introducing phenol and its derivatives into environment. Due to their versatile nature, these compounds are widely used in various pharmaceutical, pulp and paper, pesticides, dyes, leather, and paint industries (Sandhibigraha et al., 2019; Khleifat, 2006). In addition, these are used to manufacture disinfectants, wood preservatives, insecticides, and adhesives. These are also released during the incineration of the municipal waste, chlorination of the drinking water, smoking, and vehicles exhaust to the environment (Anku et al., 2017; Yang and Lee, 2008). Sharma and Gupta (2012) have reported that the paper and pulp mill effluent contains phenol up to 200 mg/L whereas 50 - 500 mg/L of phenolic compounds were found in the refinery and coke oven industries effluent (Nayak and Singh, 2007; Pattanaik et al., 2018).

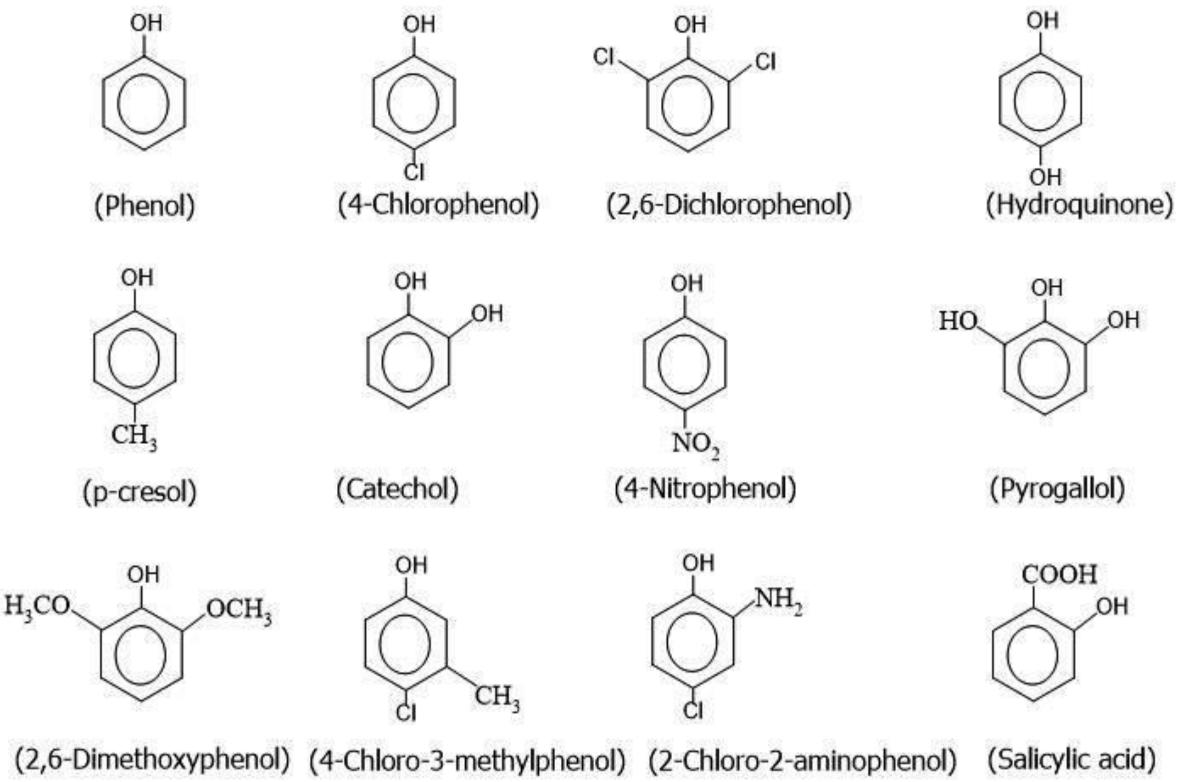

Figure 1. Chemical structures of phenol and some of its derivative compounds.

1.1.2. Toxicity and permissible limit in the environment

The phenol and its derivatives undergo oxidation to form more toxic compounds such as quinones and semiquinone radicals which can damage the DNA and cellular proteins (Schweigert et al., 2001). A chronic exposure to these compounds may damage the internal organs such as the liver, heart, and kidney. Moreover, it causes skin irritation, muscle weakness, and vomiting under acute exposure to human beings. The compounds such as para-cresol and alkylphenols can induce carcinogenic and teratogenic effects in human beings (Paisio et al., 2012; Anku et al., 2017). The toxicity of these compounds has been studied by taking a broad group of microorganisms, algae, and aquatic animals. However, the value of median lethal concentration (LC_{50}) was found to be in the range of 1.4 – 3.88 mg/L for crustaceans, algae, and bacteria (Duan et al., 2018). Park et al. (2012) have investigated the effect of phenol on *Lemna paucicostata* and reported that the frond growth was severely affected at a very low concentration of phenol. The acute exposure of 4-chlorophenol (4-CP) causes bioaccumulation, DNA damage, and mortality of the aquatic animals (Farah et al., 2004). Hence, phenol and its derivatives in the ecosystem may lead to a health disaster for human beings and aquatic lives. Therefore, the Bureau of Indian Standards (BIS) has set a permissible limit of phenolic concentration in drinking water as 0.002 mg/L (Seth et al., 2013). The World Health Organization (WHO) and European countries have constrained the concentration of phenolic compounds as 1 and 5 μg/L, respectively, in drinking water (Srivastava et al., 2006; Schummer et al., 2009).

1.2. Treatment methods

The concentration of phenol and its derivatives has been found more than the permissible limit in the river and lakes (Seth et al., 2013; Singh et al., 2020). As evident from the above, the presence of phenol and its derivatives in the environment can cause significant deterioration of health and even fatal to human beings, aquatic culture, and microorganisms. Therefore, the phenolic concentration in the wastewater should be lower than the permissible limit set by the regulatory authorities. The persistent nature of the phenol and its derivatives makes the researchers develop economical and effective methods for their degradation. The development and use of various treatment techniques were based on cost-effectiveness, reusability, eco-friendliness, sludge generation, and handling operation. In this direction, physical, chemical, advanced oxidations, and biological techniques were extensively studied. A summary of advantages and disadvantages of these techniques is shown in Table 1.

1.2.1. Physical methods

Generally, the physical method includes adsorption, coagulation, and membrane separation technologies. Coagulation is a traditional method used to remove the phenol and its derivatives in wastewater (Golbaz et al., 2014). However, the shortcomings of this technique include longer duration and post-treatment requirements of the wastewater. Therefore, several works have been reported to increase biodegradation performance using a hybrid process involving coagulation and other techniques (Ye et al., 2021; Ogando et al., 2019).

The adsorption method involves the molecular force of attraction between the pollutant molecules and the solid adsorbent. The difference of distribution coefficients between the adsorbent and the pollutant phase is the driving force for the separation. This method is simple, easy, and cost-effective. Activated carbons, produced from the carbonization of various natural wastes (plant residue and agricultural waste), polymers (chitosan, sodium alginate), clay, and zeolites, have excellent adsorbing properties due to the high specific surface area, pore-volume, and low cost. The adsorption technique has been widely investigated to remove phenol and its derivatives and found that high removal efficiency could be achieved within a short retention time period (Zhang et al., 2020; Bazrafshan et al., 2016). Moreover, the enhancement of adsorption ability has been carried out by developing synthetic adsorbents and carbon nanotubes, which ultimately increases operation cost (Han et al., 2020; Nguyen et al., 2013; Vieira et al., 2020). Nevertheless, higher degradation efficiency, the regeneration of the adsorbents, which causes secondary waste generation, is a major limitation of this method (Singh et al., 2020; Paisio et al., 2014).

The membrane-based method is reliable and effective for the removal of phenol and its derivatives due to less power consumption and negligible secondary sludge generation (Raza et al., 2019; Muñoz et al., 2016). According to the pore size of the membrane, the above method includes various processes such as microfiltration, ultrafiltration, and nanofiltration. In addition, other methods such as membrane bioreactor, membrane distillation, emulsion liquid membrane, and pervaporation are popular among researchers. However, the high cost, fouling, and stability issues of the membrane are the major concern for the applicability of this technique on a large scale (Ortega et al., 2017; Vieira et al., 2020).

1.2.2. Chemical and advanced oxidation methods

Chemical oxidation methods involve complete oxidation of the phenol and its derivatives in the presence of various oxidizing chemicals and catalysts at mild pressure and temperature (Mohammadi et al., 2014). The reagents and chemicals used in this method are chlorine and metal cations (Cu^{2+}, Fe^{2+}, Co^{2+}, Mn^{2+}, etc.). The main disadvantage of this method includes high chemical consumption (Ramírez et al., 2017; Singh et al., 2017). The electrochemical oxidation process is an effective method as it requires no additive chemicals and increases the reaction rate. However, this method further increases the operating cost due to energy and equipment expenses (Singh et al., 2020).

Advanced oxidation methods are categorized into various techniques such as ozonation, UV/H_2O_2 treatment, Fenton, and photocatalytic oxidation. The mechanism of these methods includes the formation of free radical ion ($OH^{\cdot}$), which completely degrade the phenol and its derivatives. Although these methods are highly effective for wastewater treatment, high fixed cost, secondary waste generation, high operation skill requirement, and generation of toxic byproducts formation are the major drawbacks (Diya'uddeen et al., 2011; Ahmed et al., 2010).

1.2.3. Biological methods

Besides the various physical, chemical, and advanced oxidation methods, the biological processes are more favorable due to eco-friendly nature, low cost, and no or less harmful byproduct formation (Ho et al., 2009). The elimination of toxic intermediates, ease of operation, and cost-effectiveness are the key advantages of the biological methods over other conventional methods (Mohanty and Jena, 2017; Panigrahy et al., 2020). In this method, the degradation of phenolic compounds is carried out by using bacteria, yeast, or fungi (biodegradation), and algae (phytodegradation) (Umamaheswari et al., 2020; Paisio et al., 2014). *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Ralstonia*, and *Acinetobacter* sp. are the most popular bacterial species to degrade phenol and its derivatives (Khalid and El-Naas, 2012; Al-Zuhair and El-Naas, 2012; Singh et al., 2017). Similarly, various fungi (*Amorphoteca* sp., *Neosartorya* sp., *Talaromyces* sp., and *Graphium* sp.), yeast (*Candida sp.*, *Yarrowia* sp., and *Pichia* sp.) genera were utilized for the mineralization of the phenol and its derivatives to simpler substances like CO_2 and H_2O (Singh et al., 2017; Basak et al., 2019; Martínková et al., 2016; Priyadharshini and Bakthavatsalam, 2016). The biological methods are quite effective for detoxifying phenol and its derivatives; therefore, the treatment of phenol and its derivatives containing wastewater by biodegradation has been mainly focused in this work.

Table 1. Summary of advantages and disadvantages of various methods used for wastewater treatment.

Types of methods	Examples	Advantages	Disadvantages
Physical	Adsorption	High capacity	Secondary waste generation
	Coagulation	Simple	High chemical cost
	Membrane separation	High efficiency	High pressure, expensive
Chemical	Chlorination	Inexpensive	Toxic to humans
	Oxidation (using metal cations) and electrochemical oxidation	Less time required	High chemical and energy cost
Advanced oxidation	Ozonation	Complete elimination	Short half-life, highly toxic
	Fenton	Applicable to soluble and insoluble dyes	Requirement of acidic media
	UV/H_2O_2	Used in drinking water treatment system	UV light penetration can be obstructed by turbidity
	Photocatalytic oxidation	No foul formation	Secondary sludge generation
Biological	Biodegradation, phytodegradation, vermidegradation	Eco-friendly, Ease of operation, cost-effective	Slow process, depends on environmental factors

1.3. Factors affecting biodegradation of phenol and its derivatives

The various external factors affect the biodegradation of phenol and its derivatives, including pH, temperature, dissolved oxygen (DO) availability, and concentration of phenol and its derivatives (Khalid and El-Naas, 2012). They control the biodegradability of these compounds by hindering or stimulating the growth of bacteria. Therefore, the optimization of the critical factors should be carried out to get maximum removal efficiency.

1.3.1. Effect of pH

The pH plays a vital role during the biodegradation of phenol as it controls the metabolic activity of the bacterial cell. Most bacterial species can tolerate a pH value range from 6.0-8.0. A pH value below 6.0 or above 8.0 induces more undissociated proton or hydroxyl radicals in the surrounding liquid. The electrostatic charge of the bacterial cell cannot prevent them from entering into the cell membrane (Rajani and Reshma, 2016). In most research articles, the optimum pH was reported to be 7.0 for the maximum biodegradation of phenol and its derivatives. Mohanty and Jena (2017) have investigated the effect of pH on biodegradation of phenol using *Pseudomonas sp.*, varying the pH from 6.0 – 8.0. They have reported that the phenol biodegradation was decreased with the variation of pH from either side of 7.0. The maximum removal efficiency was obtained at a neutral pH. Similarly, Bera et al. (2017) have observed that the specific growth rate of the bacteria is adversely affected at high acidic or basic conditions, and the optimum growth rate was obtained at a pH of 6.5.

1.3.2. Effect of temperature

Temperature is a crucial factor in biodegradation as it affects the metabolic activity of microorganisms. Phenol and its derivatives biodegradation occur mainly in the range of 20 – 30 °C. In addition, at a temperature above 30 °C, the phenol degradation decreased due to the death of the bacterial cell (Rajani and Reshma, 2016). This phenomenon implies that the biodegradation of phenol is a temperature-dependent process. The literature review suggests that the bacterial growth rate becomes double with the increase of temperature by 10 ℃ in the mesophilic range of 10 - 30 ℃ (Basha et al., 2010). No bacterial growth impact was observed within the temperature range of 35 – 40 ℃. However, above 40 ℃, the bacterial growth was adversely affected due to the denaturation of cellular protein and nucleic acids. Exposure to a high temperature may cause damage to the cell membrane and cell wall of the bacteria. However, several research works have been conducted to investigate the biodegradation of phenol and 4-CP by thermophilic bacteria under extreme temperatures. Ali et al. (1998) have reported the biodegradation of phenol by *Bacillus* strains at a temperature range of 50 – 60 ℃. Backman and Jansson (2004) have investigated a comparative analysis of the metabolic activity of *Arthrobacter chlorophenolicus* A6 for biodegradation of 4-CP at temperatures of 5 and 28 ℃. They found that the microbial growth rate and metabolic status of the cells were lower in 5 ℃ compared to 28 ℃. Hence, the optimum temperature should be maintained during the biodegradation of phenol and its derivatives for maximum degradation of the pollutants. The optimum temperature mainly falls within the range of 30 – 40 ℃ and 50 – 60 ℃ in the mesophilic and thermophilic environments, respectively (Fang et al., 2006; Basha et al.,2010; Singh et al., 2020).

1.3.3. Effect of dissolved oxygen concentration

The aerobic bacteria utilize molecular O_2 as a co-substrate and intercellular H_2 for its metabolic respiration (Zou et al., 2018). The oxygen acts as a final electron acceptor during the biodegradation of phenol and its derivatives. The dissolved oxygen (DO) plays a crucial role in the bacterial growth rate and the biodegradation of phenol and its derivatives. Several research works have been investigated to evaluate the critical limit of DO for optimum aerobic respiration and biodegradation rate (Basha et al., 2010). The critical DO level in the wastewater was reported to be 0.5 mg/L for flocculent microbial species (Rajani and Reshma, 2016). For an instance, the specific growth rate of bacteria and biodegradation of 2, 4-dichlorophenoxyacetic acid is inhibited with the decrease of DO. Zou et al. (2018) have reported that the biodegradation rate of phenol drastically decreased by 89 % when DO levels went down from 5 mg/L to 0.5 mg/L.

1.3.4. Effect of phenolic concentration and other carbon sources

Apart from pH, temperature, and DO, the concentration of phenolic compounds also affect biodegradation. The bacterial activity is inhibited at a high concentration of phenolic compounds due to its toxic nature (Arutchelvan et al., 2006; El-Naas et al., 2009). Various techniques were employed, such as the acclimatization of bacterial cells at high phenol concentration, immobilization, and developing genetically modified microorganisms to avoid toxicity. In addition, the substrate inhibition effect can be minimized by adding carbon and nitrogen sources as co-substrates (Khleifat, 2007). Several researchers have reported the enhancement of biodegradation of phenolic compounds in the presence of glucose, peptone, yeast, dextrose, etc. (Loh et al., 2000; Sahikaya and Dilek, 2006). Glucose is a monosaccharide carbohydrate that can easily be broken down by bacteria for their metabolic activity. It has been observed that the addition of glucose up to 0.8 g/L increases the biomass density, results in higher biodegradation of phenol using *Bacillus* sp. CDQ (He et al., 2013). If the glucose concentration is further increased, the inhibition effect was observed due to competition among the substrates. Glucose can also be an excellent co-substrate for the biodegradation of 4-CP by using *Pseudomonas putida* (Tarighian et al., 2003).

1.3.5. Effect of nutrients

For the optimal metabolic respiration of bacteria, supplementary nutrients such as Ca, Na, K, Fe, Mg, N, P, and S are essential, along with carbon sources (Agu et al., 2017; Haleem et al., 2003). The addition of nutrients in the wastewater enhances the microbial activity and biodegradation efficiency. The nutrients act as electron acceptor (Fe^{2+}), metallic enzyme activator (Cu^{2+}, CO^{2+}), and enzyme stimulator (Ni^{2+}), which improve the biomass density and removal of the pollutants (Burgess et al., 1999). The composition of the essential nutrients like N, P, and K varies along with the types of wastewater. However, various researchers have optimized the composition of nutrients for the biodegradation of aromatic and petroleum wastewater (Singh et al., 2020; Vyas and Dave, 2010). However, an excess concentration of nutrients causes a toxic effect on bacterial diversity (Chaillan et al., 2004). It also decreases flocculation and nitrification of wastewater (Oudot et al., 1998; Burgess et al., 1999). Therefore, an optimal value of nutrients should be added to the wastewater for maximum biodegradation of phenol and its derivatives.