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# **Chapter 1: Introduction**





## 1.1. Introduction

In the last few decades, the available clean water has been contaminated due to rapid population expansion, urban development, and industrial growth (Jiang et al., 2015). These activities resulted in the generation of a wide range of complex pollutants. Specifically, the pollutants released from industrial activities, such as dyes and phenolic compounds, have sparked significant concerns among both the scientific community and the public due to their toxic, cancer-causing, and mutagenic characteristics (Jun et al., 2019). The most prevalent phenolic compounds present in wastewater are phenol, cresol, 2,4,6-trichlorophenol, 2,4-dinitrophenol, and others. Various industries, including chemical, textile, petrochemical, pesticide, pharmaceutical, petroleum, and tannery sectors, collectively produce approximately 700 million tons of phenolic compounds each year (Ren et al., 2017). Over 10 million tons of phenolic compounds are estimated to be discharged into the environment (Alshabib and Onaizi, 2019). The phenolic compounds are highly soluble in water, so lowering their concentration to meet safety standards (ranging from 0.1 to 1 mg L<sup>-1</sup>) poses challenges (Vaiano et al., 2018). Typically, petrochemical industries (2.8 to 1220 mg L<sup>-1</sup>), coal mining operations (9 and 6800 mg L<sup>-1</sup>), coke oven plants (28 to 1200 mg L<sup>-1</sup>) and petroleum oil refineries (6 to 500 mg L<sup>-1</sup>) are the primary sources for discharging phenolic compounds into the surrounding aquatic environment (Panigrahy et al., 2022a). Phenolic compounds are considered hazardous chemicals, and chronic exposure to these substances can impact human well-being. These cause symptoms like headaches, diarrhoea, skin irritation, loss of appetite, and gastrointestinal distress, as acute exposure to phenolic compounds can be fatal, resulting in harm to the nervous system, heart, kidneys, and liver in humans (Jun et al., 2019).

Due to their toxic nature, these compounds have been categorized in the priority pollutant list by the United States Environmental Protection Agency (USEPA). Furthermore, the USEPA has established a water purification standard aiming for a concentration of less than 1 ppb of phenolic compounds in surface waters (Busca et al., 2008).

## 1.2. Impacts of phenolic pollutants on the environment

Numerous phenolic compounds are crucial in industrial processes, with their usage being indispensable for humans. However, because of their high stability, the natural biodegradation of these compounds takes several decades, making them difficult to degrade rapidly. The difference between the generations of phenolic pollutants and the duration needed for their elimination causes these substances to steadily build up in the environment,

leading to irreversible outcomes. Phenolic compounds have the ability to disperse across gaseous and condensed phases, perpetually entering the natural aquatic environment through direct discharge, rainfall, and runoff from agricultural sludge. Consequently, they are extensively distributed throughout the air, clouds, rain, fog, and snow (Wu et al., 2022). As phenolic pollutants possess greater density than water, they tend to settle at the base of the water body. Also, it is reported that more than 1 ppm of phenolic compounds in aquatic environments exert an adverse impact on marine life (Biglari et al., 2017).

Prolonged and consistent exposure to phenolic substances, even at low levels, can lead to their accumulation within organisms, disrupting the endocrine system and impacting hormonal balances in aquatic organisms and wildlife (Sakhi et al., 2018).

The levels of the majority of identified phenolic pollutants found in samples collected from various regions exceeded the threshold limit set by the World Health Organization (WHO) for phenolic compounds in drinking water (Ramos et al., 2021). Conventional treatment facilities for drinking water are not specifically engineered to eliminate trace organic compounds (TrOCs), making most TrOCs resistant to conventional purification methods for drinking water (Chen et al., 2021). This circumstance heightens the likelihood of phenolic substances persisting in drinking water. The potential hazards associated with the accumulation of phenolic substances in the environment have emerged as a global concern.

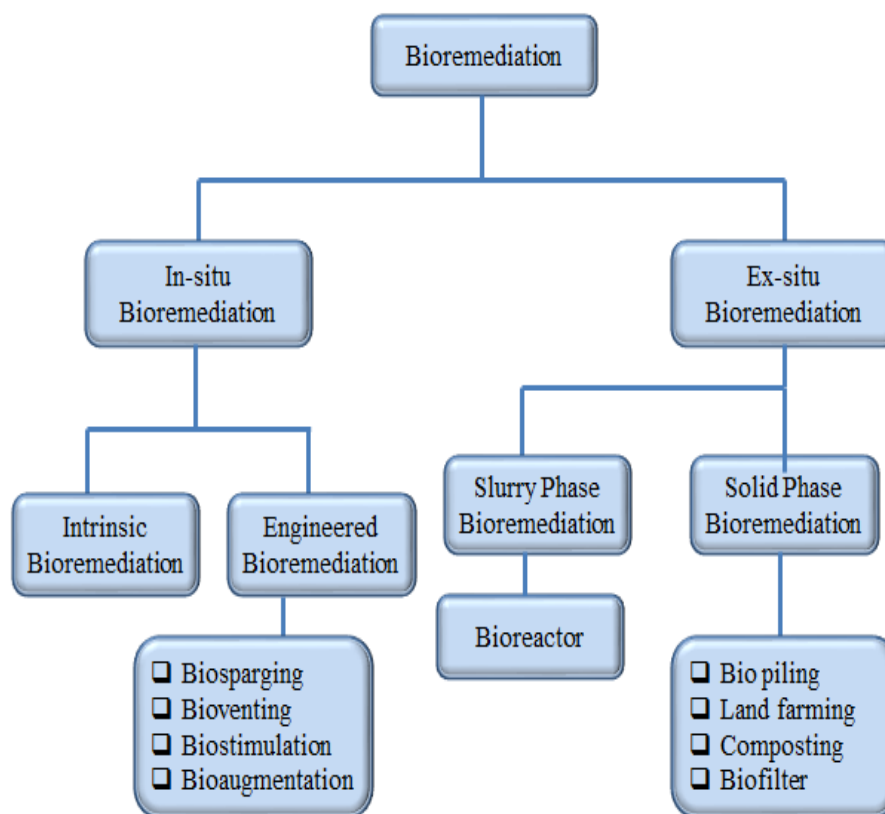
### 1.3. Available technology for the removal of phenolic compounds

Numerous methods have been explored to treat wastewater containing phenolic compounds, including adsorption, advanced oxidation, photocatalysis, electrolysis, and membrane-based approaches (Mahdavianpour et al., 2018). The substantial drawbacks of these methods include their high expenses, the creation of secondary harmful substances, and their lack of sustainability (Panigrahy et al., 2020). Among the various technologies (Fig. 1.1.), bioremediation technology is considered as the most suitable approach for eliminating organic pollutants at reduced expenses (Tian et al., 2020). Therefore, it presents an appealing method for eliminating the phenolic compounds from wastewater to meet effluent standards at a lower cost.

### 1.4. Biological methods for removal of phenolic compounds

The bioremediation technique has surfaced as a promising approach for completely degrading phenolic pollutants into either CO<sub>2</sub> and H<sub>2</sub>O or harmless products through mineralization. Varieties of algae (*Scenedesmus*, *Chlorella*, *Chlorella vulgaris*, etc.), yeast strains (*Candida*

*oregonensis*, *Candida subhashii*, *Schizoblastosporion Starkeyi-henricii*, etc.), fungi (*Ascomycetous*, *Aspergillus fumigates*, *Debaryomyces*, *Aspergillus niger* and *Basidiomycetes* species), bacterial strain (*Pseudomonas sp.*, *Pseudomonas aeruginosa* and *Klebsiella variicola* strains) have been tested for the treatment of wastewater containing phenolic pollutants (Panigrahy et al., 2020, Mahgoub et al., 2023). It is an environmentally friendly method for managing organic waste and mitigating environmental pollution. Biological treatment can be divided into aerobic and anaerobic degradation. Aerobic biodegradation is widely utilized in wastewater treatment plants, composting facilities, and natural ecosystems to break down organic waste pollutants. In contrast, anaerobic biodegradation occurs without oxygen and is typically used to decompose organic matter in environments like landfills and specific wastewater treatment processes. Based on oxygen availability, biodegradation can be classified as *In-situ* and *Ex-situ*.



**Fig. 1.1. Different technologies available for biodegradation**

#### 1.4.1. *In-situ* bioremediation techniques

These methods address the contaminated substances (pollutants) directly where they are found, avoiding the need to move pollutants from their original location. Consequently, these approaches are expected to be more cost-effective than ex-situ bioremediation methods since

they eliminate expenses associated with excavation. However, there is a noteworthy concern regarding the costs of designing and installing advanced on-site equipment to enhance microbial activities during bioremediation (Azubuïke et al., 2016). *In-situ* bioremediation techniques are classified as (i) intrinsic and (ii) engineered bioremediation.

### **(i) Intrinsic bioremediation**

Intrinsic bioremediation, also known as in-situ natural reduction or passive bioremediation, operates without external intervention (human involvement), allowing polluted sites to be remediated passively. This technique involves activating the indigenous or naturally existing microorganisms in a contaminated site (Yaashikaa and Kumar, 2022). However, a significant limitation of this approach is the potential for an extended duration required to achieve the desired pollutant remediation level, as it doesn't involve human interventions to accelerate the process (Patel et al., 2022).

### **(ii) Engineered bioremediation**

In this alternate method, humans intervene by introducing microorganisms to enhance the bioremediation rate. By altering the physicochemical conditions to optimize growth and employing genetically modified microorganisms, the bioremediation technique significantly speeds up the process of natural biodegradation (Patel et al., 2022). Enhance bioremediation is of many kinds: (a) Biosparging, (b) Bioventing, (c) Biostimulation, (d) Bioaugmentation,

#### **(a) Biosparging**

Biosparging is a method that entails introducing air into the soil beneath the surface to enhance microbial activity, promoting the elimination of pollutants from contaminated areas. Its goal is to facilitate the movement of pollutants from saturated zones to unsaturated zones by injecting air. This process ensures the even distribution of volatile compounds from highly saturated areas to lower ones, effectively encouraging biodegradation (Kao et al., 2008). There are several difficulties with this method when it comes to distributing soluble nitrate (to create an anaerobic environment) and air/O<sub>2</sub>/H<sub>2</sub>O<sub>2</sub> (to create an aerobic environment). However, the treatment expenses vary significantly based on the types of pollutants, their concentrations, utilization of electron acceptors, and the rate of groundwater pumping (Sravya and Sangeetha, 2022).

#### **(b) Bioventing**

In bioventing, oxygen is introduced into unsaturated areas to enhance the activity of native microbes, thereby improving the biodegradation rate. It involves supplementing nutrients and

moisture to further enhance the bioremediation process. In this process, the organic contaminants in the unsaturated zone may be more easily converted by bacteria into less harmful forms (Sun et al., 2023). The treatment cost significantly fluctuates based on soil type and surface area (Saroj et al., 2022).

### **(c) Biostimulation**

In biostimulation, the remediation process is initiated by encouraging the proliferation of indigenous bacteria already present in contaminated areas. This is achieved by introducing different types of nutrients that limit growth rates, including phosphorus, nitrogen, oxygen, carbon, and electron acceptors (Höhener and Ponsin, 2014). Biostimulation is one of the most effective techniques for hydrocarbon bioremediation (Patel et al., 2022).

### **(d) Bioaugmentation**

When bioattenuation and biostimulation have failed, it is believed that bioaugmentation strategy should be used (Hassan et al., 2023). Potential microbes are added to supplement the existing microbial population in this biodegradation process. Pollutant-degrading bacteria (indigenous and non-indigenous) use intracellular enzymes that allow them to transform hydrocarbons into another food source (Sayed et al., 2021). This technique offers several advantages, such as enhancing effectiveness and accelerating the decomposition of substances while decreasing pollutant levels.

## **1.4.2. *Ex-situ* bioremediation techniques**

These methods involve the extraction of pollutants from contaminated sites and their transfer to a different location for treatment. Various factors come into play when evaluating *ex-situ* bioremediation techniques, such as treatment expenses, the depth and nature of contamination, and the extent and site of pollution. Performance criteria determine the choice of the most suitable *Ex-situ* bioremediation technique among these methods. *Ex-situ* technique is classified as (i) Slurry phase and (ii) Solid phase bioremediation.

### **(i) Slurry phase bioremediation**

Slurry-phase bioremediation is a relatively fast process compared to alternative treatment methods. It involves mixing pollutants with water, nutrients, and oxygen to establish the ideal conditions for microorganisms to break down contaminants. The specific amounts of added water, nutrients, and oxygen are tailored to the pollutant concentration and biodegradation rate. This process creates an optimal environment within the vessel, often referred to as a bioreactor.

### (a) Bioreactors

These are widely used as a vessel for managing bioremediation in controlled slurry conditions. Operating based on the pollutant's characteristics; these can function in batch, fed-batch, sequencing batch, multistage, or continuous modes. However, it requires significant initial capital investment. However, it provides regulated pH, temperature, mixing, aeration, substrate, and inoculum ratios, and so on. It protects against external influences like rain and unpredictable weather like extreme temperatures. Each bioreactor design caters to distinct needs and drawbacks. Hence, selecting the right design according to specific requirements enables the optimal utilization of its benefits (Patel et al., 2022). Based on different operational modes, various types of bioreactors are available, such as batch and continuous, featuring diverse designs like Airlift, Continuous Stirred Tanks, Fluidized Beds, Trickle-Bed, Packed-Bed Biofilm, and Membrane Bioreactors.

#### a.1. Fluidized bed bioreactors

The biological removal of organic and inorganic environmental pollutants is frequently hindered by factors such as low concentrations of contaminants, their toxicity, and the limited energy yields attained from the degradation of pollutants by the microorganisms driving the reactions. Since the 1980s, there has been a growing utilization of fluidized bed bioreactors (FBRs) as a successful technology in the treatment of water and wastewater. FBRs (Fig. 1.2.) involve the suspension of solid biomass carriers by employing high liquid or gas flow rates, enabling these carriers to fluidize and exhibit fluid-like behaviour.

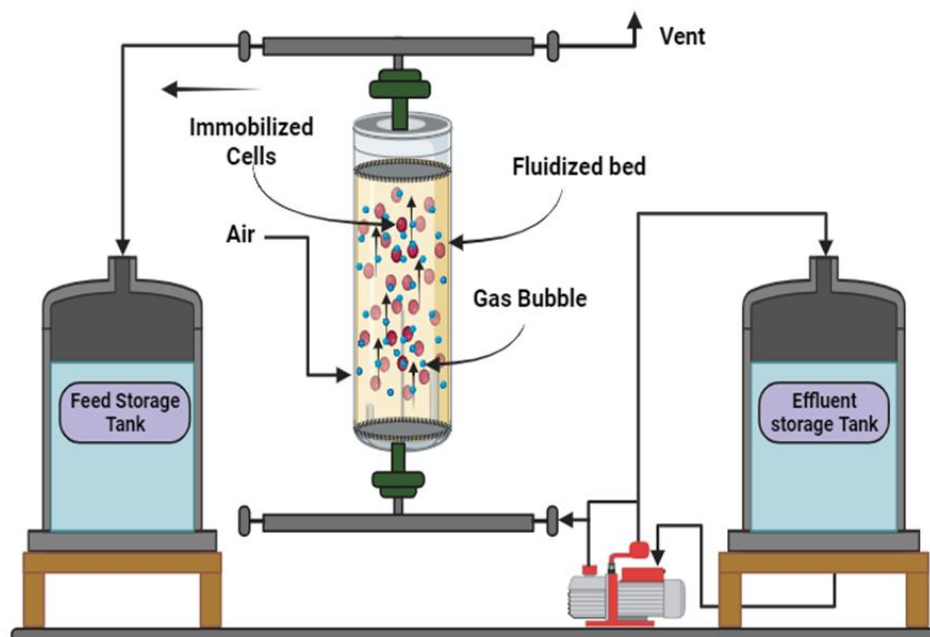


Fig. 1.2. Systematic diagram of fluidized bed bioreactor

FBRs were created for the purpose of treating wastewater; however, this technology possesses numerous other potential applications beyond wastewater treatment. This technology has found extensive use in environmental engineering across various applications, such as treating household wastewater, breaking down persistent organic compounds, reducing or oxidizing organic and inorganic pollutants, facilitating the bio-precipitation of diverse inorganic compounds through oxidation or reduction reactions (Özkaya et al., 2019). Nevertheless, drawbacks linked with FBRs involve expenses related to pumping to sustain the upward velocity required for bed expansion and fluidization. The power essential for the recycling pump must surpass the combined frictional resistance and density variations between the fluid and the expanded bed (Özkaya et al., 2019).

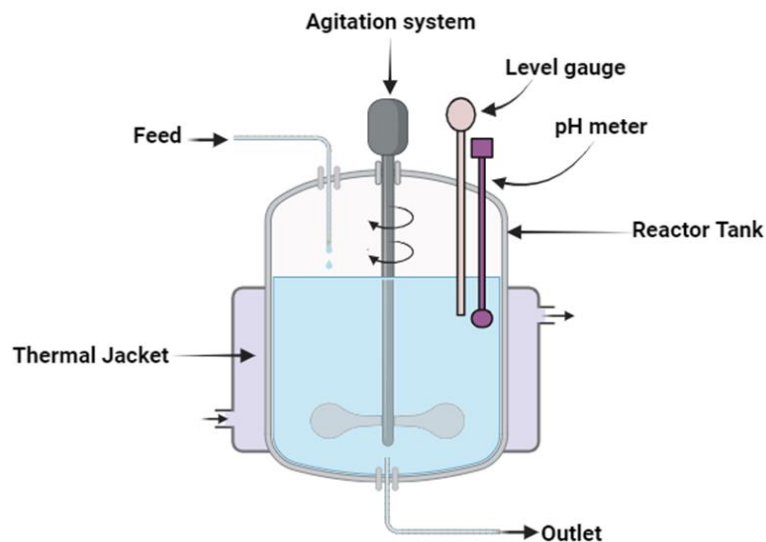
The Environmental Protection Agency (EPA) of the United States outlined four primary drawbacks of FBRs in 1993 such as:

- ❖ Dimensional constraints on the reactor to maintain the height-to-diameter ratio
- ❖ The energy requirements involved with maintaining extremely high recycling rates
- ❖ The difficulties in regulating biomass levels and selecting the proper medium
- ❖ Challenges in maintaining precise process control due to complexities in monitoring biomass concentration

Furthermore, liquid distributors for introducing influent into the reactor can be expensive in full-scale systems. A considerable period is necessary for the formation of biofilm during start up, and the uniform fluidization might be impeded by clogging in the flow distributor (Rabah and Dahab, 2004).

## a.2. Continuous stirred tank bioreactor

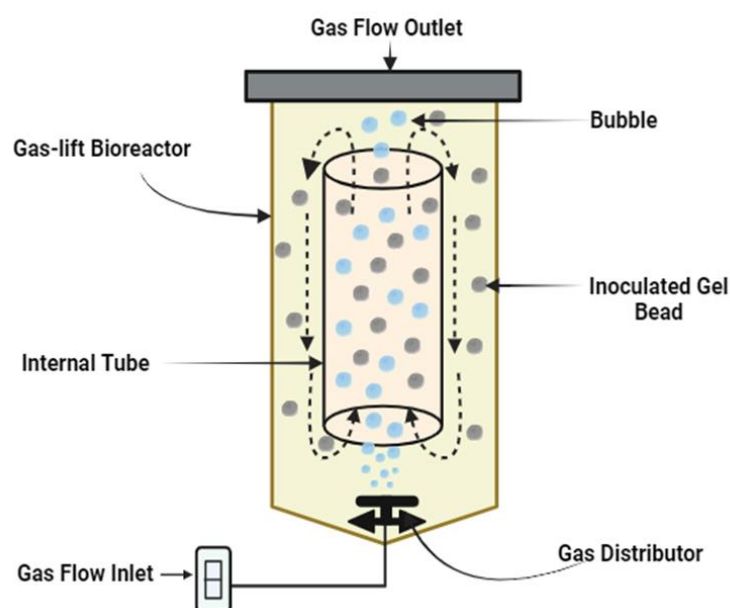
A continuous stirred tank bioreactor (CSTBR, (Fig. 1.3.)) was employed to refine a viable and dependable bioprocessing system for treating pollutants in industrial wastewater. CSTBR offers practical benefits, including straight forward scalability, enhanced fluid mixing, efficient oxygen transfer capabilities, and the availability of diverse impellers suitable for handling liquids ranging from moderate to high viscosity (Kariyama et al., 2018, Tomei et al., 2021, Saravanan et al., 2023). These reactors demonstrate a high degree of responsiveness to operational factors, encompassing temperature, pH, and hydraulic retention time (HRT) (Banu et al., 2021). However, a drawback of CSTBR in achieving perfect mixing is the escalation in expenses due to higher power consumption. Additionally, the shear stress imposed on immobilized cells as the impeller initiates mixing can severely damage shear-sensitive cells (Tiwari et al., 2023).



**Fig. 1.3. Systematic diagram of continuous stirred tank bioreactors**

### a.3. Airlift bioreactor

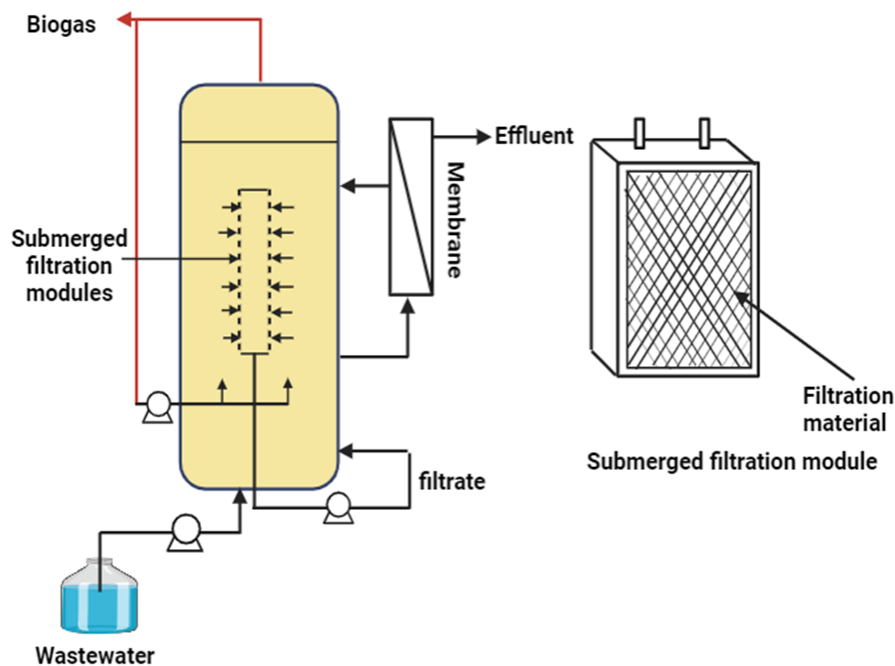
The airlift bioreactor (ALBR, (**Fig. 1.4.**)) is a gas-liquid contact device extensively used in industrial wastewater treatment. In contrast to stirred tank bioreactors, airlift bioreactors minimize power usage and cellular shear stress while enhancing mixing, mass transfer, and heat transfer coefficients. Additional benefits include a simple design without moving components, controlled flow, effective mixing, clearly defined residence times for all phases, and cost-effective oxygen transfer ([Shokrkar et al., 2018](#)). ALBR can be effectively improved by ensuring the provision of nutrients and oxygen to microorganisms through the retention of oxygen bubbles ([Asadi et al., 2017](#)). Conversely, the downside of airlift bioreactors is their increased initial capital cost, primarily attributed to large-scale operations.



**Fig. 1.4. Systematic diagram of airlift bioreactor**

#### a.4. Membrane bioreactor

Smith et al. introduced the MBR technology in 1969 through the Dorr-Oliver research program. However, it remains an evolving and emerging technology for wastewater treatment. MBRs (Fig. 1.5.) demonstrate superior treatment efficacy compared to other biological systems because of their exceptionally dense microbial population near the membrane surface. This proximity ensures comprehensive pollutant removal before the wastewater undergoes filtration through the membrane (Goswami et al., 2018).



**Fig. 1.5. Systematic diagram of membrane bioreactor**

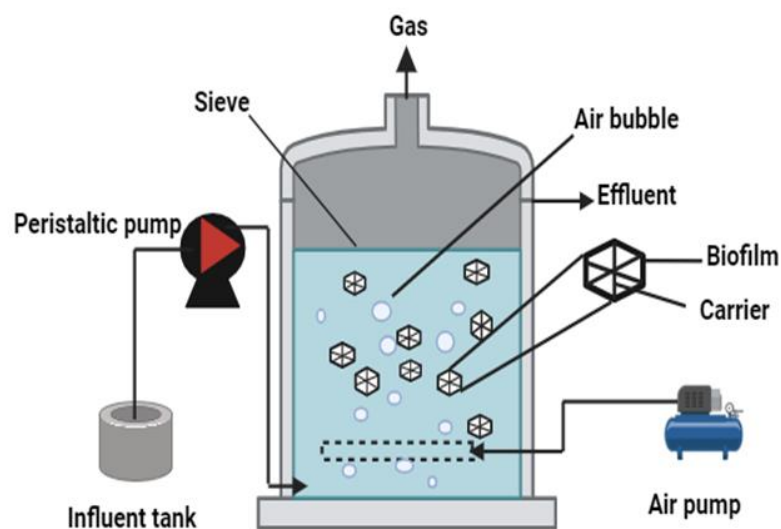
Additionally, MBR serves as an alternative to the conventional activated sludge (CAS) treatment, replacing the clarifier with a membrane to address settling issues when undesired biomass forms. Furthermore, because of the membrane's sieving action, pollutants with a molecular weight surpassing the membrane's molecular weight cut-off are retained. This retention brings these pollutants into contact with the degrading microorganisms within the MBR, ensuring their thorough degradation (Ahmed et al., 2017). On the contrary, apart from the elevated expenses associated with MBR implementation, membrane fouling emerges as a significant issue. Fouling not only affects the microbes within the reactor but also deteriorates the membrane. Additional concerns include limitations regarding pH, temperature, pressure, and certain corrosive chemicals (Mutamim et al., 2013).

Different aeration approaches classify membrane bioreactors. In order to treat the wastewater, both Aerobic Membrane Bioreactors (AMBRs) and Anaerobic Membrane Bioreactors (AnMBRs) are employed, with the former being prevalent in extensive-scale applications.

Anaerobic membrane bioreactors (AnMBRs) have also become a favourable substitute for aerobic wastewater treatment methods. AnMBRs require less energy input and generate lower sludge quantities compared to aerobic processes. Additionally, using AnMBRs reduces the required operational space and the quantity of unit operations compared to conventional processes (Maaz et al., 2019). The AnMBR technology is well-suited for treating diverse streams, particularly wastewater from food industries and municipalities (Lin et al., 2013).

### a.5. Moving bed biofilm reactor

The fundamental concept behind the moving bed biofilm reactor (MBBR, (Fig. 1.6.)) process involves the development of biomass attached to media known as carriers. These carriers remain in motion within the bioreactor, propelled either by diffusers in aerobic bioreactors or by mechanical stirrers in anoxic or anaerobic bioreactors. The movement is essential in the bioreactor to facilitate the transfer of substrates to the attached biomass within the biofilm and to maintain a thin biofilm layer through shearing forces. MBBR fulfils criteria such as minimal head loss, a substantial specific surface area, resistance to clogging, and the ability for uninterrupted operation (Leyva-Díaz et al., 2017).



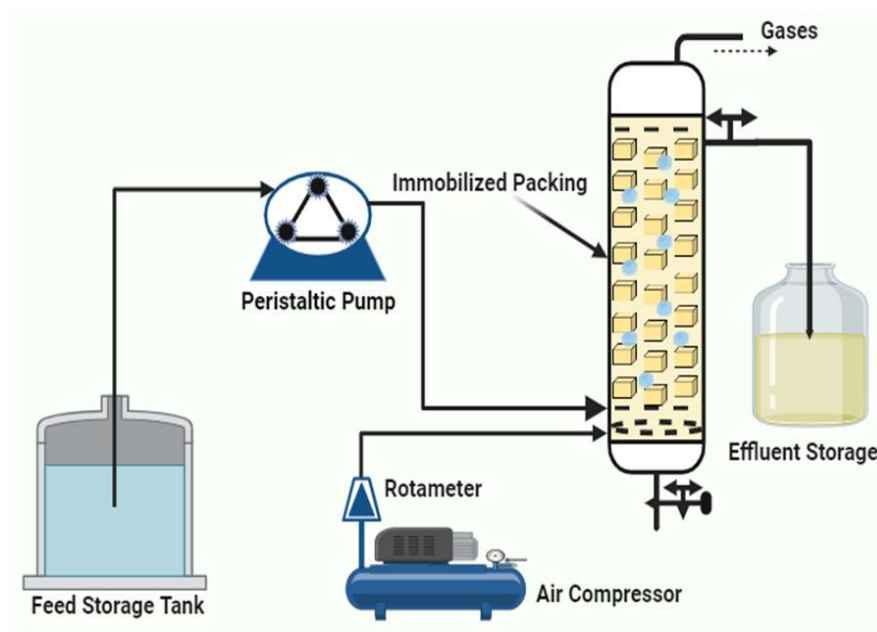
**Fig. 1.6. Systematic diagram of moving bed biofilm reactor**

The effectiveness of a treatment can be influenced by the shape, dimensions, and surface area of the media used (Iannacone et al., 2021). The design of the carrier can impact biofilm thickness, penetration, and diffusion. Employing an appropriate carrier can aid in sustaining optimal nutrient and oxygen levels within the colonies attached to it, fostering the development of a thin biofilm on the surface despite any turbulence. It has been effectively applied in treating both industrial effluent and domestic wastewater.

### a.6. Packed bed bioreactor

Recently, significant attention has been directed towards packed bed bioreactors (PBBR) to eliminate pollutants from wastewater. PBBRs exhibit superior performance efficiencies compared to traditional technologies utilizing suspended cultures (Dizge et al., 2011). The advantages of PBBRs employing attached microbial cells include operational versatility and the substantial volumetric productivity of microbial cells. This productivity is achieved and sustained over prolonged periods due to the stable microenvironment provided by the carrier for the microorganisms. The bio-affinity, structure, and chemical composition of the biocarrier are pivotal in the biodegradation process.

Consequently, substantial focus has been placed on creating cost-effective, porous, and long-lasting biocarriers. Various materials, including activated carbon, sugarcane bagasse, low-density polyethylene, calcium alginate, polyacrylamide, and polypropylene, have been employed to immobilize microorganisms in pursuit of this goal. Various reports indicate that polyurethane foam (PUF) is a high-grade carrier for cell immobilization due to its exceptional chemical resilience, elevated porosity, and stability. PUF provides a substantial specific surface area conducive to microorganism attachment, consequently amplifying biodegradation. A schematic diagram depicted in **Fig. 1.7.** illustrates the layout of a PBBR, comprising a cylindrical column featuring an air inlet, a feed liquid inlet, a distribution space at the base, and air outlets and treated liquid at the apex. This setup is supported by an abundance of inert solids known as column packing and is commonly utilized for bioremediation wastewater.



**Fig. 1.7. Systematic diagram of packed bed bioreactor**

[Sahoo et al. \(2016\)](#) conducted a study investigating the degradation of 4-chlorophenol (4-CP) using *Arthrobacter chlorophenolicus* A6 within a packed bed reactor. They achieved nearly complete degradation of 4-CP at a loading rate of  $1707 \text{ mg L}^{-1} \text{ d}^{-1}$ , along with a 97.9% removal of toxicity. However, when the pollutant loading rates surpassed  $1707 \text{ mg L}^{-1} \text{ d}^{-1}$ , the performance of the packed bed reactor system declined due to the transient accumulation of toxic intermediates like 4-chlorocatechol. Furthermore, the bioreactor system exhibited resilience against high shock loading conditions.

[Geed et al. \(2017\)](#) utilized PUF to immobilize *Bacillus sp.*-S4 for the biodegradation of Malathion in both batch and continuous packed bed bioreactors (PBBR). In the batch PBBR, after a span of 10 days, 89% removal of malathion was recorded. Operating the continuous PBBR at various flow rates ( $5$  to  $30 \text{ mL h}^{-1}$ ) under optimal conditions for 75 days achieved inlet loading rates of  $36$  to  $216 \text{ mg.L}^{-1}.\text{day}^{-1}$  and elimination capacities of  $7.20$  to  $145.4 \text{ mg L}^{-1} \text{ day}^{-1}$ , with maximum removal efficiency 90%.

## **(ii) Solid phase bioremediation**

### **a. Biopiling**

Biopiles-based bioremediation entails piling contaminated soil above ground, then enriching it with nutrients and potentially aerating it to boost microbial activity. Its key elements include aeration, watering, nutrient supply, leachate collection, and a treatment platform. This off-site method is gaining popularity due to its cost-efficient nature, fostering effective degradation provided nutrient levels, temperature, and aeration remain well regulated ([Azubuiké et al., 2016](#)). In this technique, the irrigation/nutrient setup is buried beneath the soil to facilitate the passage of air and nutrients through either vacuum or positive pressure. Soil mounds, reaching heights of 20 feet, might be wrapped in plastic to manage runoff, evaporation, and volatilization while encouraging solar heating.

### **b. Land farming**

Land farming is one of the more straightforward bioremediation methods due to its cost-effectiveness and minimal equipment demands for operation. It is commonly classified as an ex-situ bioremediation method; however, it is also considered an in-situ bioremediation technique in certain instances ([Azubuiké et al., 2016](#)). The primary goals of this approach are to stimulate microorganisms and accelerate the aerobic breakdown process. It is often used to clean up places polluted with hydrocarbons, notably polyaromatic hydrocarbons. [Wang et al. \(2016\)](#) and [Rozaimah et al. \(2020\)](#) researched enhanced land farming techniques for addressing soil contamination caused by diesel and lubricant oil. They introduced several

additives: compost, activated sludge sourced from oil-refining wastewater facilities, nutrients, fern chips, and TPH (Total petroleum hydrocarbon) degrading bacteria to augment TPH removal. Incorporating compost and activated sludge was observed to elevate soil microorganism populations and enhance degradation efficiency, achieving an 83% removal rate over a 175-day treatment period.

### **c. Biofilter**

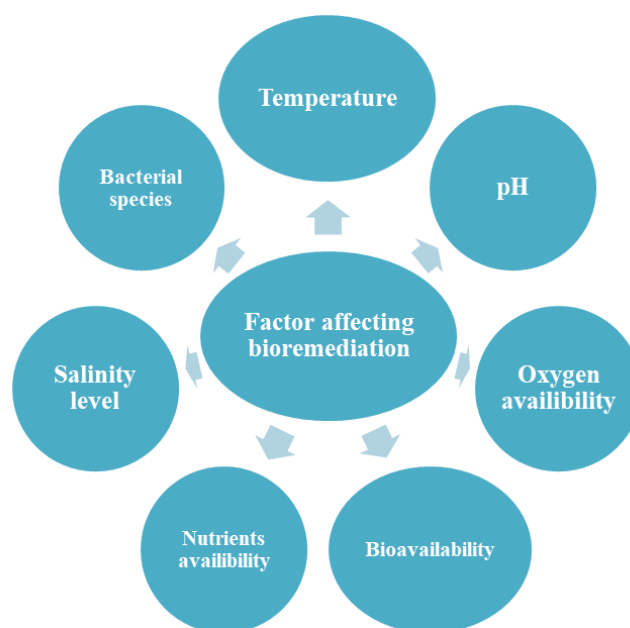
In biofiltration, gaseous organic pollutants like hydrocarbons are directed through a soil bed, where they adhere to the soil surface and get broken down by soil microorganisms. These filters might contain specific bacterial strains that specialize in breaking down specific compounds. Biofilters offer various benefits over traditional activated carbon absorbers ([Patel et al., 2022](#)). Biofilters sustain their maximum adsorption capacity by self-regeneration, which is their standout advantage. Their primary benefit lies in the destruction of pollutants as opposed to mere separation ([Saravanan et al., 2015](#)). Historically, biofilters were unable to handle chlorinated compounds effectively. However, recent demonstrations have proven their capability to remove such compounds. When an excess of bacteria is present, the filters require periodic cleaning through mechanical means.

### **d. Composting**

Composting bioremediation refers to utilizing and customizing composting techniques for managing waste and addressing contaminants ([Sayara and Sánchez, 2020](#)). It involves an aerobic method that relies on oxygen, ideal moisture levels, and permeability to break down organic waste effectively. Its key regulating factors include temperature, oxygen levels, and moisture content. This technique has been utilized for bioremediation purposes in soils tainted with substances such as petroleum hydrocarbons, solvents, chlorophenols, pesticides, herbicides, polycyclic aromatic hydrocarbons, and nitro-aromatic derivatives ([Dzionek et al., 2016](#)).

## **1.5. Factor affecting biodegradation**

In bioremediation, different contaminants from their environment are removed or detoxified using microorganisms, including bacteria, algae, fungi, and plants. The enzymatic metabolic pathways of microorganisms accelerate the breakdown of pollutants by biochemical processes. Certain environmental factors influence the enzymatic activity of the microorganisms (**Fig. 1.8**).



**Fig. 1.8. Factors affecting biodegradation process**

### **(i) Temperature**

The significance of temperature in bioremediation cannot be overstated. It substantially impacts microbial growth rate, the solubility of gases, soil composition, microbial metabolism, and contaminants' physical and chemical condition (Varjani and Upasani, 2017). Temperature variations can lower the viscosity of pollutants, leading to improved diffusion rates of these pollutants, resulting in them being easily accessible by microorganisms. Typically, degradation slows down in colder conditions. As temperature drops, pollutants' viscosity increases, lowering their ability to dissolve and providing bacteria with a non-soluble carbon source. Additionally, temperature significantly influences bacterial metabolism. As enzymes are protein-based, lower temperatures impede bacterial metabolic functions, while moderate temperature increases can enhance them. The ideal temperature for isolated bacteria to biodegrade pollutants (such as hydrocarbons) was observed within the range of 30 °C to 40 °C (Bera et al., 2019a; Singh et al., 2022b; Zdarta et al., 2018).

### **(ii) pH**

Variations in wastewater pH can impact how effectively phenolic biodegradation occurs, affecting both the solubility of phenolics and the physiological functions and enzymatic activities of microorganisms. In acidic pH conditions, phenolic compounds exist in a non-ionized, hydrophobic form, easily penetrating microbial cell membranes and exacerbating their toxicity (Panigrahy et al., 2022). Nevertheless, in highly alkaline pH conditions, microbial growth and the rate of phenolic degradation decrease, likely attributed to the adverse effects of phenolics on the activities of phenol oxidase and peroxidase enzymes.

[Sivasubramanian and Namasivayam \(2015\)](#) employed a microbial consortium to break down 1000 mg L<sup>-1</sup> phenol under ideal culture conditions of 35 °C and pH 7, achieving complete removal within 96 hours. Additionally, elevating the pH had a detrimental impact on phenol biodegradation, reducing its efficiency and hindering the process. The pH between 6.0 to 8.0 is appropriate for most phenolic degrading bacterial strains ([Li et al., 2019a](#))

### **(iii) Oxygen availability**

Oxygen concentration has been identified as the primary limiting factor for the degradation of environmental pollutants ([Al-Hawash et al., 2018](#)). Aerobic microorganisms primarily utilize oxygen as the final electron acceptor in aerobic respiration. Furthermore, the microbial breakdown of diverse organic compounds, like hydrocarbons and aromatic ring compounds necessitates molecular oxygen as a co-substrate. Bacterial respiration seems unaffected beyond a specific dissolved oxygen concentration. [Basha et al. \(2010\)](#) highlighted critical dissolved oxygen levels ranging between 0.01 to 0.038 mg L<sup>-1</sup> for various yeast and bacterial cultures.

### **(iv) Bioavailability**

Bioavailability refers to the quantity of a substance accessible to microorganisms on a physicochemical level. The accessibility of contaminants to degrading microorganisms is a pivotal factor directly impacting the effectiveness of biological treatment. Bioremediation relies on the metabolic capacity of microorganisms to detoxify or alter pollutant molecules, a process heavily reliant on bioavailability ([Megharaj et al., 2011](#)). Sometimes, when employing predominant degrading strains in optimal environmental settings, the degradation efficiency might still be influenced by the proportion of contaminants accessible to the strain ([Hu et al., 2023](#)). However, pollutants with limited bioavailability, which may be caused by high hydrophobicity, low concentrations, slow diffusion rates, and poor water solubility, can sometimes impede biodegradation ([Kebede et al., 2021](#)). The biodegradability of hydrocarbons can be ranked as linear alkanes > branched alkanes > low-molecular-weight alkyl aromatics > monoaromatics > cyclic alkanes > polyaromatics > asphaltenes ([Varjani, 2017](#)).

### **(v) Nutrients**

Nutrients are essential components for successful pollutant biodegradation, including nitrogen, iron, and phosphorus in some cases ([Al-Hawash et al., 2018](#)). Some of those nutrients can become a limiting factor, thus impacting the biodegradation processes. Carbon comes from an organic source; hydrogen and oxygen are supplied from the water. On the

other hand, the concentration of excess nutrients can also inhibit biodegradation activity (Kalantary et al., 2014). Bera et al. (2019b) used common nutrients for the effective biodegradation of *p*-cresol, such as NaNO<sub>3</sub>, Na<sub>2</sub>HPO<sub>4</sub>, KH<sub>2</sub>PO<sub>4</sub>, MgSO<sub>4</sub>·7H<sub>2</sub>O, CaCl<sub>2</sub>·2H<sub>2</sub>O, FeSO<sub>4</sub>·5H<sub>2</sub>O, CuSO<sub>4</sub>·5H<sub>2</sub>O, Na<sub>2</sub>MoO<sub>3</sub> and MnSO<sub>4</sub> amended with of yeast extract.

#### (vi) Salinity level

Saline water and hypersaline water are characterized as waters with elevated concentrations of dissolved salts, primarily NaCl, surpassing 1% to 3.5% (w/v), respectively (Li et al., 2019b). It was observed that the degradation pathways and enzymes involved in the aerobic metabolism of hydrocarbon compounds are usually reported for many non-halophile bacteria. However, scarcity of information concerning the specific pathways and enzymes responsible for breaking down hydrocarbons in environments characterized by high salinity (Li et al., 2019c). Increased water salinity leads to high osmotic pressure (Imron and Titah, 2018) that disrupts the internal osmotic equilibrium of cells and inhibits bacteria's ability to produce macromolecules, affecting the efficiency of enzymes involved in the biodegradation process. Additionally, elevated water salinity results in reduced oxygen availability for microorganisms, thereby slowing down the rate of bacterial growth (Imron et al., 2020). Kee et al. (2015) showed that under salinity levels below 3%, bacteria were able to degrade hydrocarbon compounds >50%, whereas in salinity >3%, the percentage of diesel biodegradation was <30%.

#### (vii) Bacterial species

Eliminating phenolic pollutants through microbial means offers a promising strategy for purging and neutralizing hazardous substances within polluted surroundings. Various microorganisms, including fungi, yeasts, microalgae, and bacteria, effectively eradicate phenolic pollutants such as phenol, chlorophenol, nitrophenol, alkyl phenol, ethylates, and cresols. A variety of microorganisms such as *Achromobacter sp.*, *Pseudomonas sp.*, *Acinetobacter sp.*, *Bacillus sp.*, *Gulosibacter sp.*, *Arthobacter sp.*, *Halomonas sp.*, *Acinetobacter sp.* (SA01), *Achromobacter sp.*, *Rhodococcus sp.* (Rucká et al., 2017) have used for the biodegradation of phenolic pollutants from different polluted waters. Bera et al. (2019a) listed some bacterial species that degraded *p*-cresol, such as *Gliomastix indicus* MTCC 3869, *Arthobacter sp.* W1, *Bacillus sp.* PHN 1, *Stenotrophomonas maltophilia* KB2, *Phanerochaete chrysosporium* BKM-F-1767, *Gliomastix indicus* MTCC 3869, *Advenella sp.* LVX-4, A co-culture of *Rhodococcus erythropolis* M1 and *Pseudomonas fluorescens* P1, *Scedosporium apiospermum*, *Stenotrophomonas sp.* (MF004205).