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# BACTERIAL DEGRADATION OF PHENOL: A REVIEW OF THE CURRENT STATE OF KNOWLEDGE

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## ABSTRACT

Phenol is a toxic and recalcitrant contaminant widely used in various industries, including petrochemical, pharmaceutical, and agrochemical industries. Bacterial degradation of phenol is a promising method for treating phenol-contaminated wastewater. The biodegradation of phenol by bacteria can effectively remove it from the environment, making it a valuable alternative to traditional chemical treatment methods. This review summarizes the current knowledge on the bacterial biodegradation of phenol, including those in the petrochemical, pharmaceutical, and agrochemical sectors, the metabolic pathways involved in phenol degradation, and the factors that influence the efficiency of phenol biodegradation. This paper also discusses the challenges and limitations of using bacteria for the biodegradation of phenol, including the need for further research to improve the efficiency and sustainability of the process. The outcome of this review demonstrates that bacterial biodegradation is a promising and effective method for treating phenol-contaminated wastewater and provides a foundation for future research to improve the efficiency and continuous use of this process.

**Keywords**: Bacterial degradation, phenol, bioremediation, organic pollutants.

## INTRODUCTION

Phenol is an organic compound widely used in industrial and commercial applications (Figure 1). It is a colourless liquid with a distinctive, pungent odour and is highly soluble in water (Hanafi and Sapawe, 2020). Phenol is used to produce various chemicals, including plastics, resins, and fertilizers. It is also a raw material for phenolic compounds such as phenol-formaldehyde resins, including industrial and consumer products, such as adhesives, coatings, and moulding compounds. Additionally, phenol produces pharmaceuticals, herbicides, and pesticides (Kabbour and Luque, 2020).

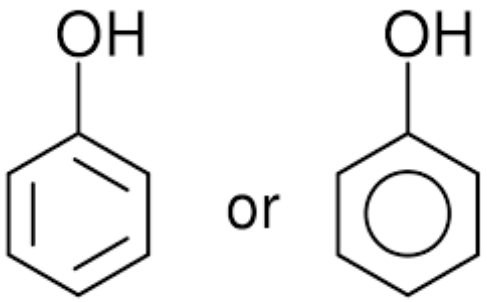


Figure 1: Chemical structure of phenol (Sobiesiak, 2017)

Due to its bactericidal properties, phenol is used in producing various consumer products, such as disinfectants, soaps, and toiletries. In addition, phenol is an antiseptic and anaesthetic in the medical field, particularly in dentistry (Tschersich *et al.*, 2021). Due to its wide application and potential to contaminate the environment through industrial processes and consumer products, it is essential to understand the biodegradation process of phenol by microorganisms in the environment, which can help mitigate the impact of phenol pollution.

### Environmental concerns associated with phenol

Phenol and its derivatives can have negative impacts on the environment. Phenol is toxic to aquatic life and can harm fish, amphibians, and other aquatic organisms at high concentrations. It is toxic to plants and can damage crops and vegetation (Kumar *et al.*, 2020). Phenol is also a known carcinogen (Smith *et al.*, 1989; Silva *et al.*, 2003; Schubert *et al.*, 2015) and can harm human health (Figure 2) if ingested or inhaled in high concentrations. It is grouped as a poisonous air-polluting agent by the United States Environmental Protection Agency (EPA) and a priority pollutant by the European Union (Silva *et*

*al.*, 2021).

In addition, phenol can contaminate groundwater and surface water through spills, leaks, or improper disposal of industrial waste. This trend can lead to contamination of drinking water sources and negatively impact local communities and ecosystems (Sashikesh *et al.*, 2023). Furthermore, phenol is persistent in the environment and can take a long time to degrade (Annachhatre and Gheewala, 1996). This scenario means that once it has entered the environment, it can remain there for extended periods, potentially causing ongoing damage to ecosystems and human health (Sun *et al.*, 2023).

Therefore, it is essential to have adequate measures in place to prevent and mitigate the release of phenol into the environment. These can include better regulations and control of industrial processes and the development of new bioremediation techniques to remove phenol from contaminated environments.

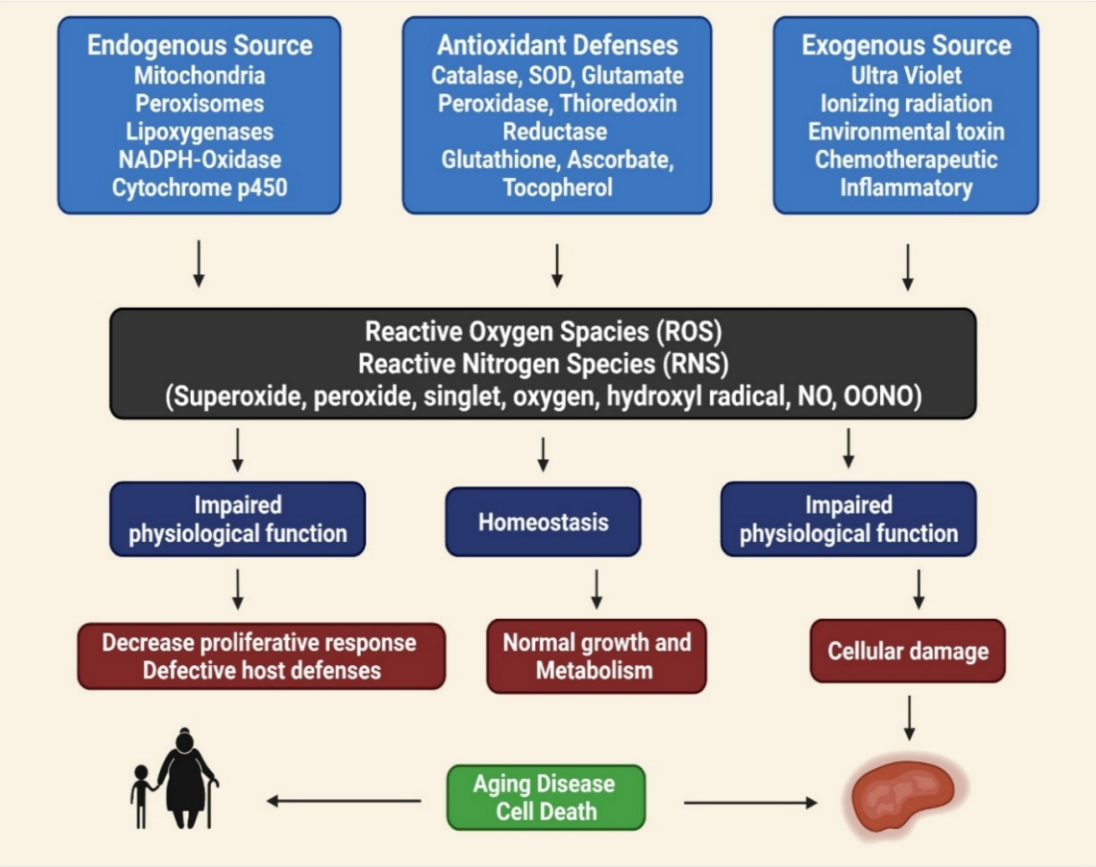


Figure 2: Role of Phenolic Compounds in Human Disease (Rahman *et al.*, 2022).

**Importance of bacterial degradation in the remediation of phenol-contaminated environments**

Bacterial degradation is a natural process that plays a critical role in the remediation of phenol-contaminated environments. Microorganisms like bacteria and fungi can break down complex organic compounds like phenol into simple, readily biodegradable compounds (Zhang *et al.*, 2023). The degradation of phenol by microorganisms can be harnessed in a process known as bioremediation (Figure 3). Bioremediation uses living organisms, typically microorganisms, to remove or neutralize pollutants from the environment (Stincone *et al.*, 2023). There are two main types of bioremediation for phenol: *in situ* and *ex-situ (*Tomei and Daugulis, 2013). *In situ* bioremediation is the process of applying microorganisms to a contaminated site, whereas *ex-situ* bioremediation is to remove the contaminated material and treat it in a separate location (Huda *et al.*, 2023).

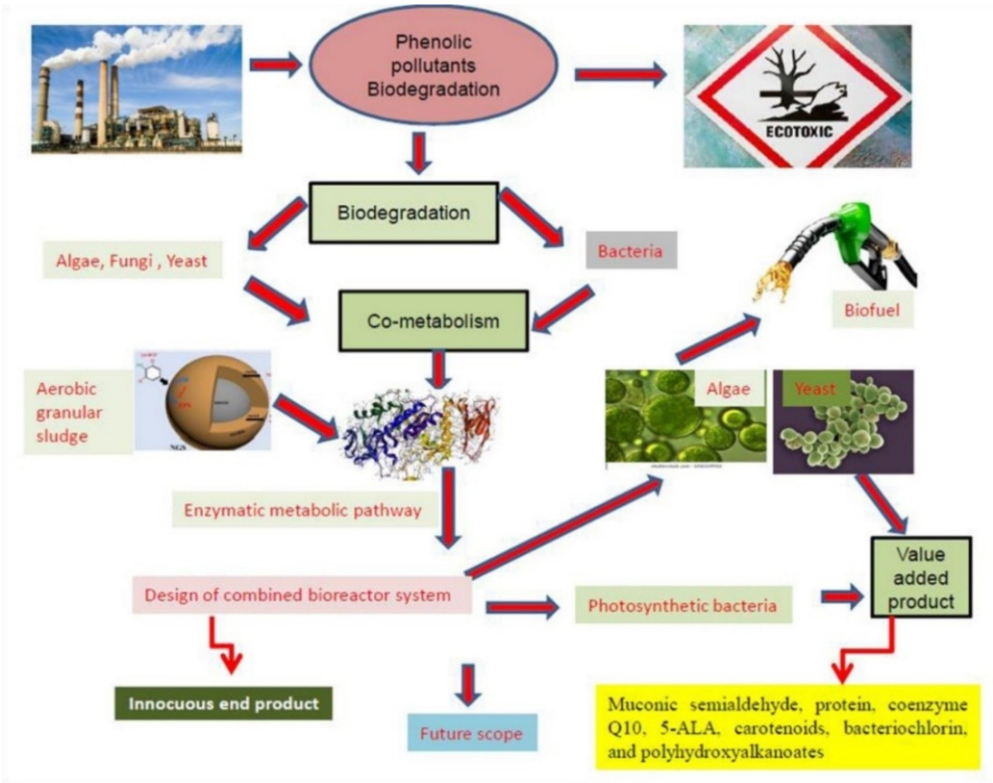


**Figure 3**: Illustration of pathway and outcome of phenol degradation by microorganisms (Panigrahy *et al.*, 2022).

Bacterial biodegradation is a cost-effective and environmentally friendly way to remove phenol from contaminated environments. It can be less expensive than traditional physical or chemical treatments and does not generate hazardous waste (El Moukhtari *et al.*, 2023). In addition, bioremediation can be more efficient than traditional methods because microorganisms can degrade pollutants *in situ*, which can be more effective and cheaper than physically removing contaminated materials. Overall, bacterial biodegradation is a vital tool in the remediation of phenol-contaminated environments, and it is an inexpensive and environmentally friendly way to remove this pollutant from the environment.

**Microbial degradation of phenol-microbial metabolic pathways for phenol degradation**

The microbial metabolic pathways for phenol degradation involve a series of enzymatic reactions (Figure 4) that convert phenol into simple, more readily biodegradable compounds. The most common pathway used by phenol-degrading bacteria is the meta-cleavage pathway, which involves the conversion of phenol into catechol, followed by the conversion of catechol into benzoate. The benzoate is then converted into an intermediate of the tricarboxylic acid cycle (Muñoz-Palazon *et al.*, 2023).

Another pathway used by phenol-degrading bacteria is the ortho-cleavage pathway, which involves the conversion of phenol into hydroquinone, followed by the conversion of hydroquinone into pyrocatechol and then into benzoate (Yang *et al.*, 2023). The catechol 2,3-dioxygenase pathway converts catechol into 2-oxo-3-hexenedioate and succinate (Han *et al.*, 2023). Lastly, some bacteria can use the phenol hydroxylase pathway, which converts phenol into phenol-2-monooxygenase and then into catechol (Yu *et al.*, 2023).

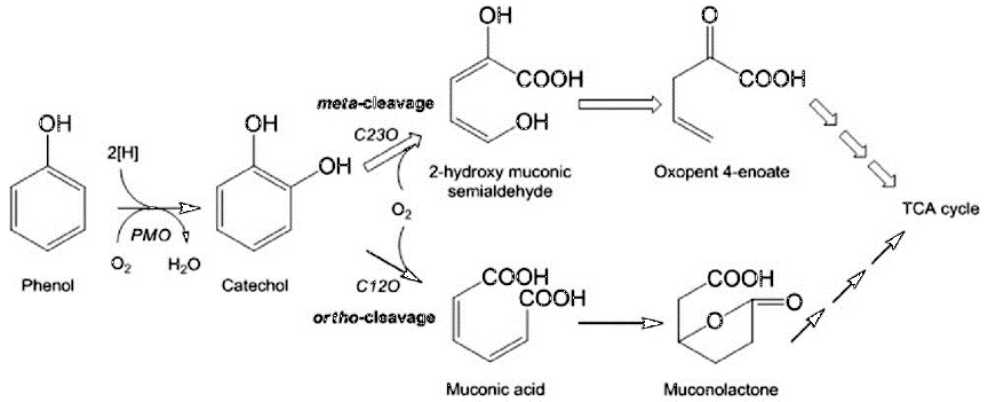


**Figure 4**: Enzymes involved in the first two steps of microbial metabolic pathways for phenol degradation (Hasan and Jabeen, 2015).

It is important to note that different microorganisms use different pathways, and it is also possible for a microorganism to use multiple pathways. The choice of pathway used also can depend on the environmental conditions and the presence of other pollutants.

## Characterization of phenol-degrading microorganisms

This section includes identifying the different types of microorganisms and their genetic makeup, metabolic pathways, and growth requirements (Yin *et al*., 2023). Microorganisms that can degrade phenol include bacteria belonging to the genera – *Pseudomonas, Alcaligenes*, and *Sphingomonas* and fungal species such as *Phanerochaete chrysosporium* and *Trametes versicolor* (El Moukhtari *et al*., 2023).

Molecular techniques such as polymerase chain reaction (PCR), deoxyribonucleic acid (DNA) sequencing, and stable isotope probing are used to identify and track phenol-degrading microorganisms in the environment (Figure 5). Techniques such as metagenomics and metatranscriptomics are also used to study the functional potential of microbial communities and identify essential genes involved in phenol degradation (Gunjal *et al*., 2023). Characterizing phenol-degrading microorganisms can help improve the efficiency and effectiveness of bioremediation strategies by providing a better understanding of the microorganisms that are most effective at degrading phenol, as well as their growth requirements and optimal conditions.

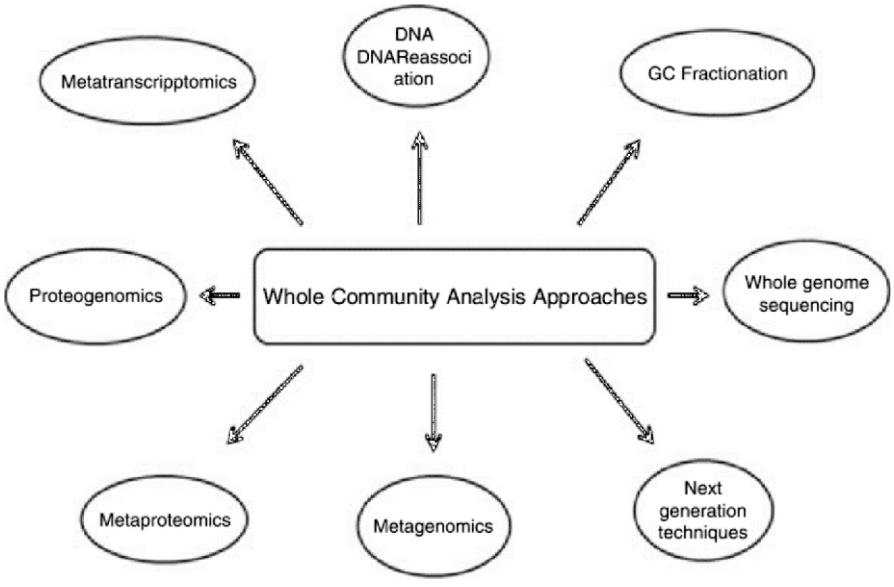


**Figure 5**: Omics technologies in studying phenol-degrading microorganisms in the environment (Biswas and Sarkar, 2018).

**Factors influencing the efficiency of phenol biodegradation**

Several factors influence the efficiency of phenol biodegradation (Yang *et al*., 2020; Wang *et al*., 2023). These include:

a. **Temperature**: Temperature directly impacts enzyme activity, microbial growth, and reaction rates in phenol biodegradation. Optimal temperatures enhance efficiency, while extremes can hinder enzymatic activity and microbial function (Alemzadeh *et al*., 2002; Haritash *et al*., 2009). Li *et al*. (2019) reported that temperature is a critical factor that significantly influences the efficiency of phenol biodegradation. The rate of microbial activity, including the processes involved in biodegradation, is highly temperature-dependent. In general, higher temperatures tend to accelerate microbial metabolic processes, thereby increasing the phenol degradation rate. However, the effect of temperature is not linear, as extreme temperatures, whether too high or too low, can inhibit microbial activity. Therefore, maintaining an optimal temperature range is essential for efficient phenol biodegradation. It's worth noting that the specific temperature range for optimal phenol biodegradation can vary depending on the microbial strains and environmental conditions, emphasizing the importance of precise temperature control in bioremediation efforts.

b**. pH:** pH plays a crucial role in phenol biodegradation. Optimal pH conditions promote enzymatic activity and microbial growth, while extreme pH values can inhibit degradation efficiency and microbial function (Sahoo *et al*., 2011; Zhao *et al*., 2020). A study noted that most microbial populations responsible for phenol breakdown have a pH range within which they function efficiently (Zhao *et al*., 2020). Many phenol-degrading bacteria, for example, flourish in slightly alkaline to neutral environments. A variation from this ideal pH range can hamper microbial activity and, as a result, phenol biodegradation effectiveness. Extreme pH levels, whether acidic or alkaline, can denature enzymes and disturb biological activities, making it critical to keep the pH range acceptable for the specific microbial strains involved in the biodegradation process.

c. **Oxygen:** Oxygen availability significantly impacts phenol biodegradation. Aerobic conditions support efficient degradation, as oxygen is a critical electron acceptor. Anaerobic conditions can impede degradation efficiency due to limited oxygen availability (Yang *et al*., 2018; Hassan *et al*., 2023). According to Liu *et al*. (2021), aerobic and anaerobic microorganisms are the two primary categories of microorganisms responsible for phenol breakdown. Aerobic microbes require oxygen to thrive, whereas anaerobic micro-organisms function without it. The presence or lack of oxygen in the environment determines which microbial group dominates, which affects phenol biodegradation efficiency. Under aerobic circumstances, when oxygen is plentiful, phenol-degrading bacteria demonstrate increased metabolic activity, resulting in faster and more efficient breakdown. However, phenol decomposition can still occur in anaerobic environments but at a slower rate.

d. **Nutrients**: Adequate nutrient availability, such as carbon, nitrogen, and phosphorus sources, is crucial for efficient phenol biodegradation. Insufficient nutrients can limit microbial growth and metabolic activity, reducing degradation efficiency (Priyadarshini *et al*., 2022; Fouad *et al*., 2023). Elmansour *et al*. (2002) reported that microorganisms involved in phenol degradation require nutrients such as carbon, nitrogen, phosphorus, and various trace elements. Adequate nutrition availability can boost the establishment of phenol-degrading microbial populations and improve their ability to break down phenol molecules efficiently. Microbial activity may be restricted in times of nutrient constraint, slowing the biodegradation process. To maximise the effectiveness of phenol biodegradation, it is critical to strike a balance by providing the appropriate number and composition of nutrients.

e. **Inhibitory compounds**: Inhibitory compounds can significantly affect phenol biodegradation efficiency. Toxic substances in the environment can inhibit enzyme activity and microbial growth, hindering the overall degradation process (Wang *et al*., 2010; Zhao *et al*., 2023). Numerous inhibiting chemicals in the environment might stymie phenol breakdown processes. Heavy metals, organic contaminants, and other harmful substances may be present in these compounds and can directly limit the growth and metabolic activities of phenol-

degrading microbes. Heavy metals, particularly mercury, cadmium, and lead, which are frequent environmental contaminants, can be harmful to microbial communities participating in biodegradation. These inhibitory substances can disturb the enzymatic processes required for phenol breakdown, potentially leading to the inactivation of phenol-degrading enzymes. As a result, in the presence of inhibitory chemicals, the effectiveness of phenol biodegradation can be greatly reduced (Meng *et al.*, 2023).

f. **Microbial community:** The composition and diversity of the microbial community play a vital role in phenol biodegradation efficiency. Synergistic interactions among different microorganisms can enhance degradation rates by sharing metabolic pathways and facilitating the removal of phenol contaminants (Zhou *et al.*, 2023). However, this is not always the case, as some microorganisms work better individually or less inefficiently in the presence of other degraders (Bouchez *et al.*, 1995).

g. **Phenol concentration**: Phenol concentration directly affects the efficiency of phenol biodegradation. Higher phenol concentrations can overwhelm microbial populations and enzyme systems, reducing degradation rates and prolonged remediation times (Rosenkranz *et al.*, 2013; Partovinia *et al.*, 2023). Tolerance to phenol concentrations varies across the microbial populations responsible for phenol decomposition. Low to moderate phenol concentrations can be biodegraded more efficiently by these microbes. However, when the concentration of phenol increases, the biodegradation rate decreases, and the process becomes less efficient. High phenol concentrations can saturate microbial populations, overloading their enzyme systems and preventing growth. As a result, keeping the phenol concentration in the environment at an ideal level is critical for successful biodegradation. A concentration that is too low may not efficiently stimulate the microbial community, while a concentration that is too high may inhibit their activity (Mahgoub *et al.*, 2023).

h. **Biostimulation:** Biostimulation techniques, e.g., adding nutrients or electron acceptors, can enhance phenol biodegradation efficiency. These strategies promote the growth and activity of phenol-degrading microorganisms, accelerating the degradation process (Chen *et al.*, 2009; Tomei *et al.*, 2021).

It is vital to note that the potency of phenol biodegradation is influenced by a combination of these factors (Figure 6) and the specific conditions of a site. Therefore, a detailed understanding of the environmental conditions and microbial community is crucial to developing effective bioremediation strategies.

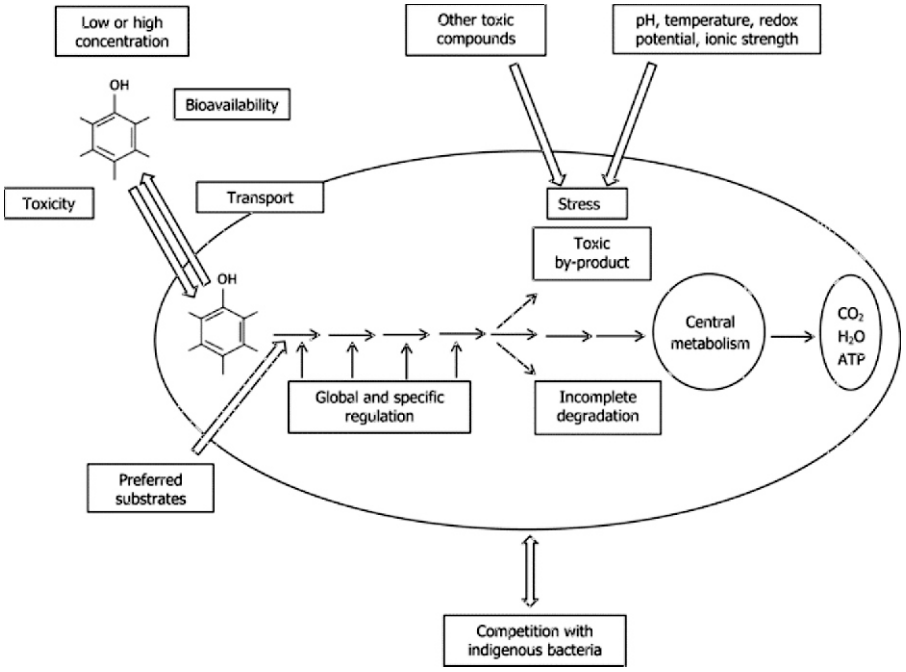


**Figure 6**: Factors influencing the efficiency of phenol biodegradation (Rucká *et al.*, 2017).

## METHODS OF STUDYING PHENOL-DEGRADING BACTERIA

Several methods are used to isolate and enrich phenol-degrading bacteria (Figure 7). These methods include:

1. **Enrichment culture**: This method involves growing phenol-degrading bacteria in a liquid medium containing phenol as the sole carbon source. The culture is periodically transferred to a fresh medium to increase the population of phenol-degrading bacteria (Maity *et al.*, 2022).
2. **Selective media**: Specialized media can isolate and enrich phenol-degrading bacteria. For example, using a medium that contains phenol as the sole carbon source can help to isolate phenol-degrading bacteria. In contrast, selective agents such as antibiotics can inhibit the growth of non-phenol-degrading bacteria (Sachan *et al.*, 2019). The growth media used to study phenol-degrading bacterium isolated from activated sludge in an industrial wastewater treatment plant were MP medium and mineral salts (Geng *et al.*, 2006).
3. **Stable isotope probing**: This technique involves growing microorganisms in a medium containing a stable isotope-labelled phenol version. The microorganisms that degrade the labelled phenol can then be isolated and characterized (Salah *et al.*, 2020).
4. **PCR-based methods**: PCR-based methods such as PCR-DGGE, PCR-TTGE, and FISH (Fluorescent *in situ* hybridization) can identify the phenol-degrading bacteria in a sample (Xie *et al.*, 2021). In a study by Gu *et al.* (2016), PCR-DGGE was used to isolate phenol-degrading bacteria from drinking water biofilters.
5. **Metagenomics:** Metagenomics can identify and study the functional potential of microbial communities, including phenol-degrading bacteria, in a sample (Xie *et al.*, 2021). In a study by Silva *et al.* (2012), fosmid libraries derived from phenol-degrading sludge samples of metagenomic libraries were used to gain deeper insight into the complex metagenome of the phenol-degrading bacteria.

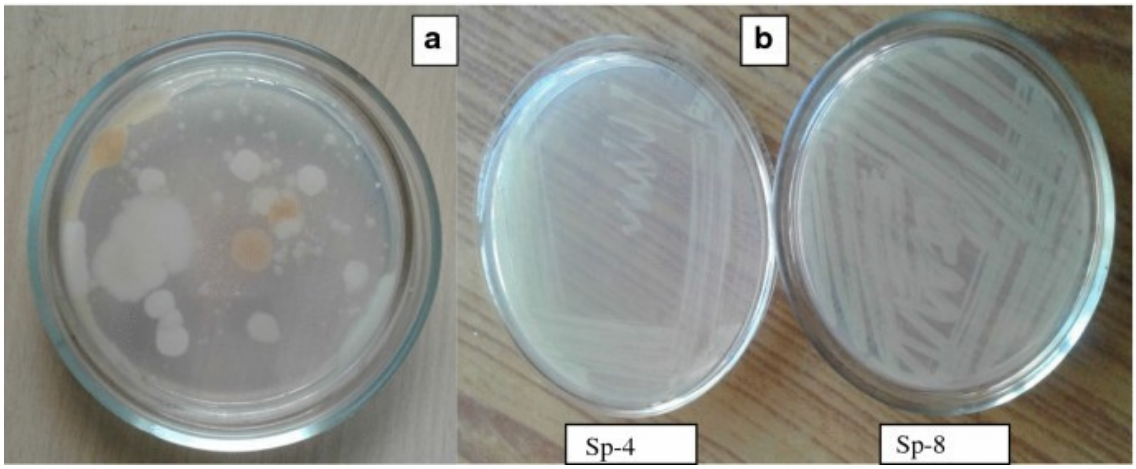


**Figure 7**: Mixed and pure cultures of phenol-degrading bacteria isolated from pulp and paper mill effluent (Sachan *et al.*, 2019).

It is paramount to note that the isolation and enrichment of phenol-degrading bacteria can be influenced by the specific conditions of a site and the presence of other pollutants. Therefore, a detailed understanding of the environmental conditions and microbial community is crucial to developing effective isolation and enrichment strategies.

### Identification of phenol-degrading bacteria using molecular techniques

Many molecular methods are used to identify phenol-degrading bacteria. These techniques include:

1. PCR-based methods: PCR-based methods such as PCR-DGGE (Denaturing Gradient Gel Electrophoresis), PCR-TTGE (Temporal Temperature Gradient Gel Electrophoresis) and FISH (Fluorescence *in situ* hybridization) can be used to identify specific genes or groups of genes involved in phenol degradation, such as catechol 2,3-dioxygenase and catechol 1,2-dioxygenase (Guerrini *et al.*, 2021).
2. DNA sequencing: DNA sequencing techniques such as 16S *rRNA* sequencing can be used to pinpoint the specific species of bacteria present in a sample (Winand *et al.*, 2019).
3. Metagenomics: Metagenomics is a technique that allows for the analysis of the entire genetic content of a microbial community. By sequencing the DNA from a community, it is possible to identify the presence of genes involved in phenol degradation and to infer the presence of phenol-degrading bacteria (Giangeri *et al.*, 2022).
4. Metatranscriptomics: This is a technique that allows for the analysis of the expression of genes within a microbial community. It enables us to infer the presence of active phenol-degrading bacteria in a sample (Agrawal and Verma, 2021).

These molecular techniques can be combined with other methods, such as growth assays, to identify and study the functional potential of microbial communities, including phenol-degrading bacteria, in a sample. It is vital to note that the identification of phenol-degrading bacteria using molecular techniques can be influenced by the specific conditions of a site and the presence of other pollutants. Therefore, a detailed understanding of the environmental conditions and the microbial community present is crucial to develop effective identification strategies.

**Functional diversity of phenol-degrading microorganisms**

Characterizing the diversity and functional potential of phenol-degrading microbial communities involves identifying and studying the microorganisms present in a sample and their ability to degrade phenol. These factors include identifying the different types of microorganisms present and their genetic makeup, metabolic pathways, and growth requirements.

Molecular techniques such as PCR, DNA sequencing, and stable isotope probing are used to identify and track phenol-degrading microorganisms in the environment. Techniques such as metagenomics and metatranscriptomics are also utilized to understand the functional potential of microbial communities and identify essential genes involved in phenol degradation (Winand *et al.*, 2019; Guerrini *et al.*, 2021).

Characterizing phenol-degrading microbial communities provides an understanding of the most effective microorganisms at degrading phenol, their growth requirements and optimal conditions. This information can improve the efficiency and effectiveness of bioremediation strategies by targeting specific microorganisms or groups of microorganisms (Rea *et al.*, 2022). Additionally, studying the microbial community's diversity can provide insight into the resilience and adaptability of the community to changing environmental conditions and perturbations. It is important to note that the characterization of phenol-degrading microbial communities can be influenced by the specific conditions of a site and the presence of other pollutants. Therefore, a detailed understanding of the environmental conditions and the microbial community present is crucial to develop effective characterization strategies (Khan *et al.*, 2019).

***In-situ* and *ex-situ* phenol bioremediation techniques**

*In-situ* bioremediation uses microorganisms to clean up pollutants in the environment where they originated from. This technique introduces microorganisms, nutrients, and oxygen to the contaminated site to break down the pollutants. *In-situ* bioremediation can be done through various methods, such as injection, bioaugmentation, and venting (Sharma, 2020; Tyagi and Kumar, 2021). Luo *et al.* (2005) used non-uniform electrokinetics to enhance in situ bioremediation of phenol-contaminated soil. The

results obtained showed that this process hastens the bioremediation process.

*Ex-situ* bioremediation is removing pollutants from the environment and treating them in a separate location. This technique involves digging up contaminated soil or water and treating it in a controlled environment, such as a bioreactor. *Ex-situ* bioremediation can be done through various methods, such as land farming, composting, and bioreactor systems (Sharma, 2020; Tyagi and Kumar, 2021). In a study on e*x situ* bioremediation of phenol-contaminated soil using polymer beads by Prpich *et al.* (2006), the microbial consortium repeatedly degraded the phenol on the polymer beads. In this study, the polymer beads were used to absorb high concentrations of phenol from the soil, thereby decreasing the initial concentration of the polluted soil.

Both *in-situ* and *ex-situ* bioremediation techniques (Figure 8) have advantages and limitations. *In-situ* bioremediation is less invasive and less expensive than *ex-situ* bioremediation, but it may not be as effective for highly contaminated sites. *Ex-situ* bioremediation can be more effective for highly contaminated sites, but it is more invasive and expensive than *in situ* bioremediation (Singh and Naaz, 2023).

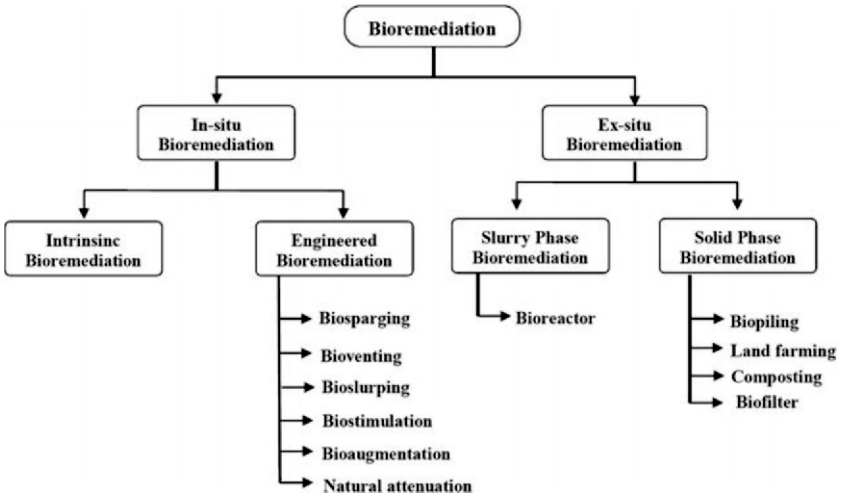


**Figure 8**: *In-situ* and *ex-situ* bioremediation techniques **(**Sharma, 2021).

**Strategies for enhancing the efficiency of phenol bioremediation**

The following methods are used to increase the efficiency of phenol bioremediation:

**Selection of appropriate microorganisms:** Using microorganisms capable of degrading phenol can enhance the efficiency of bioremediation (Jiménez-Díaz *et al.*, 2022).

**Optimization of environmental conditions:** The pH, temperature, and nutrient availability all play essential roles in the biodegradation of phenol. Optimizing these conditions can increase biodegradation rate (Al-Tarawneh *et al.*, 2022).

**Use of surfactants:** Surfactants can help solubilize phenol and increase its bioavailability to microorganisms (Zahed *et al.*, 2022).

**Addition of nutrients:** Adding nutrients such as nitrogen and phosphorus can ensure the growth and activity of microorganisms involved in the bioremediation process (Demarco *et al.,* 2023).

**Aeration:** Aeration can help provide the microorganisms with the oxygen they need to degrade phenol (Hou *et al.*, 2022).

**Bioaugmentation:** Injecting a culture of known phenol-degrading microorganisms can help speed up bioremediation (Sharma *et al.*, 2002).

**Biostimulation:** The addition of nutrients, vitamins, minerals, and other organic compounds to the contaminated site can help to stimulate the growth and activity of microorganisms involved in the bioremediation process (Adewoyin and Arimoro, 2023).

A combination of the strategies mentioned above, e.g., bioaugmentation and biostimulation, can be used to increase biodegradation rate.

### Case studies of successful phenol bioremediation projects

There have been several case studies of successful phenol bioremediation projects, including:

A study in India found that biostimulation and bioaugmentation using a mixture of bacterial strains successfully degrades phenol in a contaminated groundwater site (Manikandan *et al*., 2023). In a different study, researchers used a combination of biostimulation and bioaugmentation to treat phenol-contaminated groundwater in Iran. The treatment significantly reduced phenol concentration in the soil (Yavari-Bafghi *et al*., 2022).

A research project in Spain found that aerobic and anaerobic treatment methods effectively removed phenol from landfill leachate (Li *et al*., 2022). It is vital to note that the success of a bioremediation project depends on various factors, including the site-specific conditions and the type and concentration of pollutants present.

## CONCLUSION

Phenol is a toxic, water-soluble compound in industrial and agricultural waste streams. The biodegradation of phenol by microorganisms is a well-established process to treat phenol-contaminated water and soil. The current understanding of bacterial biodegradation of phenol suggests that various microorganisms can degrade phenol, including bacteria such as *Pseudomonas sp*., *Arthrobacter sp*., and *Rhodococcus sp*. The ability of these microorganisms to degrade phenol is due to specific enzymes, such as catechol 1,2-dioxygenase and catechol 2,3-dioxygenase, which convert phenol into less toxic compounds.

The rate of phenol biodegradation depends on various factors, including pH, temperature, nutrient availability, and the presence of other pollutants. Generally, the optimal conditions for phenol biodegradation are a neutral pH, a temperature range of 20-30°C, and sufficient nutrients, such as nitrogen and phosphorus. The bioremediation of phenol-contaminated sites can be enhanced through combined strategies, such as biostimulation, bioaugmentation, surfactant addition, and aeration.

Overall, the biodegradation of phenol by microorganisms is a potentially safe and effective treatment option for phenol-contaminated water and soil. Further research will continue to improve the understanding and efficiency of this process.

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