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Biodegradation of Phenolic Compounds in Contaminated Water: Efficacy of Immobilized Bacterial Strains and Environmental Factors

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Abstract

Water, essential for life, faces increasing contamination due to industrialization and urbanization. Phenolic compounds, persistent and toxic, are prominent pollutants in aquatic environments, necessitating effective remediation strategies. This study evaluates the degradation of phenolic compounds by two bacterial strains, *Stenotrophomonas sp.* and Pseudomonas sp. A7, focusing on their performance under varying conditions and the impact of cell immobilization on their efficacy. Phenol degradation was tested across different concentrations, temperatures, and pH levels. *Stenotrophomonas sp*. exhibited high efficiency at lower phenol concentrations, with a decrease in performance as concentrations increased. In contrast, Pseudomonas sp. A7 maintained better degradation efficiency at higher phenol levels. Optimal conditions for both strains were identified at neutral to slightly acidic pH and moderate temperatures. Immobilization of bacterial cells in calcium alginate beads improved degradation efficiency slightly, with *Stenotrophomonas* sp. achieving 93% and *Pseudomonas sp*. A7 92% removal rates. The results underscore the effectiveness of both bacterial strains in phenol bioremediation, highlighting the potential of immobilization and environmental optimization for enhancing wastewater treatment processes. Further research into bead formulation and concentration could improve the practical application of these bioremediation techniques.

Keywords: Phenol; Bioremediation; Immobilization.

1. Introduction

Water is the most valuable natural resource that exists on our planet. It covers 70% of the Earth's surface and without this invaluable compound; the life on the Earth would not exist. This is a widely recognized fact; pollution of water resources is a common problem that is being faced today [1]. With urbanization and extensive industrialization, the pollution of the environment with man-made (synthetic) organic compounds has become a major problem

The natural water bodies are contaminated by various toxic pollutants. The demand for clean and drinking water is becoming a pressing concern day by day due to the fast depletion of freshwater resources since many sources of water bodies have been exhausted because of the increasing world population and others are likely to be contaminated [2]. Hence the crisis of fresh water is being faced all over the world [3].

Industrial and domestic activities have polluted surface water as well as ground water to a greater extent [4]. Wastewater from domestic or industrial use contains waste products and severe environmental pollutants that are most often liquid or solids and are biological, chemical, or radioactive. In addition to having adverse health implications, wastewater contamination also has natural and ecological effects. Toxic wastes are being released into the environment, causing extensive environmental contamination such that many of our natural water reserves are damaged beyond repair [5]. Due to the discharge of toxic effluents long-term consequences of exposure can cause cancer, delayed nervous damage,

malformation in urban children, mutagenic changes, neurological disorders, etc [6]. Nevertheless, to ensure sustainable quality of life the environmental impact of these activities must be minimized. While conservation and better utilization of resources have the greatest influence on the sustainability of the planet; reduced generation, improved treatment technology and utilization of wastes are the best techniques for the maintenance of the environmental quality [7].

There has been heightened concern among policymakers and scientists about the effects of human and wildlife exposure to chemical compounds in the environment, particularly the aquatic environment. Phenolic compounds are among the chemicals of major concern in this regard as they tend to persist in the environment over a long period, accumulate and exert toxic effects on humans and animals [8]. Some phenolic compounds are abundant in nature and are associated with the colours of flowers and fruit [9]. Others are synthesized and are used in varied aspects of mankind's everyday life.

Phenols and their derivatives commonly exist in the environment. These compounds are used as the components of dyes, polymers, drugs and other organic substances. The presence of phenols in the ecosystems is also related with production and degradation of numerous pesticides and the generation of industrial and municipal sewages. Some phenols are also formed during natural processes. These compounds may be substituted with chlorine atoms and may be nitrated, methylated or alkylated. Both phenols and catechols are harmful ecotoxins.

Contamination of aquatic bodies by different harmful organic pollutants including phenols has stimulated research activity in the development of various treatment technologies to remove, or better to degrade, these pollutants from water and wastewater. Several chemical processes are carried out for this purpose. Generally, these technologies involve the oxidation of organic pollutants with various oxidizing agents like ozone, UV radiation, electrochemical methods, hydrogen peroxide, etc. Chlorination is not applicable, as it leads to the formation of more toxic chloroorganics [10].

The application of biotechnological processes that involve microorganisms to solve environmental pollution problems is rapidly growing. The researchers have proved that biological methodology is versatile, highly stable, has broad applications in various areas, economical and efficient for the remediation of petroleum. One of the key points for bioremediation is maintaining a high biomass of bacterial populations. To improve the survival and retention of the bioremediation agents in the contaminated sites, bacterial cells must be immobilized [12].

Among the various developed methods for cell immobilizing encapsulation in calcium alginate beads was used in this study. Alginates (polymers made of different proportions and sequences of mannuronic and guluronic acids extracted from brown algae) are easy to handle, nontoxic to humans, the environment, and the entrapped microorganisms, legally safe for human use, available in large quantities, and inexpensive [13]. From a physiological perspective, a major advantage of alginate is that immobilized cells do not suffer extreme changes in physicochemical condition during the procedure of immobilization and the gel is transparent and permeable [14]. However, this substance cannot be maintained for a long period in aqueous solution because the encapsulation immobilized microorganism can easily be broken during the operation [15].

In our study, we isolated bacteria from various polluted environments specifically to degrade phenols. We identified the optimal conditions for their activity and subsequently immobilized the bacteria to enhance their phenol degradation efficacy.

2. Materials and Methods

2.1 Samples collection

The wastewater and sediment samples were collected from the drain of different locations of some local industrial outlets which contaminated area by phenol in Alexandria, Egypt. The sediment samples were collected from 1-12 cm below the surface using a sterile knife after removal of the top layer of the soil up to 1-2 cm. Two subsamples were taken from each point and placed in a sterile plastic bag. The total mass of the soil sample collected was around 50 grams. Wastewater samples were collected from a depth of 15 cm in sterile 1000-ml bottles and transported on ice to the laboratory. The wastewater samples and sediment soil samples were collected using sterilized plastic bottles and plastic bags, allowing enough air space in the bottles and the bags for bacteriological analysis [16].

Phenol and chemicals, used in the study, were of analytical grade; inorganic salts, used in preparing microbial growth media, were of reagent grade.

2.2 Screening of Phenol Tolerating Isolates

The phenol-degrading bacterial isolates were isolated from contaminated soil and wastewater samples near some local industrial factories. In this method 5 g of each two soil samples and 10 ml of water sample (a total of three samples), each sample was added to three autoclaved 500-ml Erlenmeyer flasks containing 100 ml of MSM media. The flasks

were inoculated with 100 ppm of phenol, tightly sealed and incubated at 30◦C for 7 days under shaking conditions (150 rpm). The medium turned to a turbid solution the turbidity indicated the growth of bacteria, and then 50 µl were spread onto MSM media agar plates with 10 ppm of phenol. Then the plates were further incubated at 30◦C for 3 days. After the incubation period morphologically, different colonies were streaked on agar plates for purification before being inoculated into the liquid medium to check their degradation ability. The bacterial isolates were given a prefix of "A" followed by the number from (A1 to A9) [16, 17].

2.3 Degradation of Different Concentrations of Phenol in Liquid Medium by Isolated Bacterial Isolates

The bacterial isolates (from A1 to A9) were selected and further examined for their degradation potential on phenol. In 250 ml autoclaved Erlenmeyer flasks containing 50 ml of Mineral Salt Media (MSM) for the enrichment culture phenol was supplemented in the media as the sole carbon source, and the various concentrations of phenol were 20, 50, 75, 100, 150 mgL-1, and 4% (v/v) inoculums of each isolate separately (OD: 1.4 at 600 nm). The flasks were tightly sealed and were incubated under shaking conditions at 30◦C /200 rpm for 72 hours. Aliquots (10 ml) were taken from different flasks and centrifuged at 6000 rpm for 15 min to separate the bacterial cell mass. The supernatant was used for spectrophotometric analysis of phenol degradation percentage at 510 nm (λ max of phenol) [17].

2.4 Phenol Determination and Degradation Assay

Phenolic materials react with 4-aminoantipyrine in the presence of potassium hexacyanoferrate(III) at pH 10, adjusted using a buffer solution, to form a stable reddish-brown antipyrine dye. The colour intensity correlates with the phenolic material concentration [18].

To prepare the buffer solution, dissolve 16.9 g NH4Cl in 143 mL concentrated NH4OH and dilute to 250 mL with distilled water. This solution will adjust the pH to 10 when 2.0 mL is added to 100 mL of distillate. For the aminoantipyrine solution, dissolve 2 g of 4-aminoantipyrine (4-AAP) was prepared in distilled water and diluted to 100 ml. Similarly, potassium ferricyanide solution was prepared by dissolving 8 g of K3Fe(CN)6 in distilled water and diluting to 100 ml. Both solutions should be freshly prepared for each phenol determination.

For phenol determination, add 2 ml of buffer solution to 100 ml of distillate or an appropriately diluted aliquot and mix to ensure the pH is 10 ± 0.2. Then, add 2.0 mL of the aminoantipyrine solution and mix, followed by 2.0 mL of the potassium hexacyanoferrate(III) solution. After 15 minutes, measure the absorbance at 510 nm.

The calibration curve was prepared by plotting known concentrations of phenol versus corresponding absorbance values. Degradation activity was expressed in terms of percentage degradation and was determined by monitoring the decrease in absorbance at 510 nm (λ max of phenol) using UV-VIS PG/T60 spectrophotometer. The MSM media was used as blank; samples containing phenol without microorganisms were used as reference [19] (control) and the percentage degradation calculated by applying the following equation:

$$\text{Percentage of phenol degradation(\% PD)} = \frac{\text{Initial phenol conc(mg/L)} - \text{Residual phenol conc(mg/L)}}{\text{Initial phenol degradation (mg/L)}} \times 100 =$$

2.5 Degradation Potential of Various Phenol Concentrations in Liquid Medium by the Isolated Bacterial Strains

The degradation potential of bacterial isolates (A1 to A9) on phenol was examined. Each isolate was inoculated into 250 ml autoclaved Erlenmeyer flasks containing 50 ml of Mineral Salt Media (MSM) with phenol as the sole carbon source at concentrations of 20, 50, 75, 100, 150 mg/L, and 4% (v/v) inoculum (OD: 1.4 at 600 nm). The flasks were incubated at 30°C with shaking at 200 rpm for 72 hours. After incubation, 10 ml aliquots were centrifuged at 6000 rpm for 15 minutes to separate bacterial cell mass, and the supernatant was analyzed spectrophotometrically for phenol degradation at 510 nm. Four effective isolates (A2, A7, A8, and A9) were further tested with phenol concentrations ranging from 100 to 700 ppm under the same conditions. The degradation extent was measured after 72 hours at 510 nm using a spectrophotometer.

2.6 Molecular Characterization of the Potent Phenol-Degrading Bacteria

Molecular characterization of the most potent phenol-degrading bacterial isolate, DNA was extracted using the phenol-chloroform method. Gene amplification of 16S rRNA was performed by polymerase chain reaction (PCR) using specific primers. The partial DNA sequence of 16S rRNA was amplified, a common approach for bacterial gene identification due to its relatively small size, which facilitates faster sequence analysis (Madukasi, Chunhua et al. 2011). The specific forward primer used was 5´-AGAGTTTGATCMTGGCTCAG-3´ and the

reverse primer was 5'-TACGGYACCTTGTTACGACTT-3'.

2.7 Effects of Temperature, Medium Conditions (Static vs. Shaking), and Other Factors on Phenol Degradation

Temperature Influence: Phenol degradation was assessed at different temperatures (25°C, 35°C, 37°C, and 40°C) in a medium with a constant pH of 7. Experiments were conducted with sterilized MSM broth inoculated with bacterial isolates (4% of A2, A7, and a consortium) and 1000 mg/L of phenol. The degradation was monitored under both shaking (200 rpm) and static conditions over 72 hours. After incubation, the culture was centrifuged, and the supernatant was analysed spectrophotometrically to measure the degradation percentage.

pH Influence: The effect of pH on phenol degradation was studied in the range of pH 5 to 10. MSM media was adjusted to these pH levels using HCL or NaOH, then inoculated with 4% of bacterial isolates A2 and A7, each with an OD of 1.4 at 600 nm, and 1000 ppm of phenol. The cultures were incubated under static conditions at an optimal temperature of 37°C. Degradation efficiency was evaluated spectrophotometrically at 24, 48, and 72 hours.

Contact Time Influence: To determine the impact of contact time on phenol degradation, experiments were conducted under optimal conditions (37°C, pH 7, static conditions) with 4% of A2, A7, and their consortium, and 1000 ppm of phenol. Aliquots were taken at various intervals (3, 6, 9, 18, 21, 24, 27, 30, 33, 48, 51, 54, and 72 hours), centrifuged to separate the supernatant and analysed spectrophotometrically to assess phenol degradation over time

2.8 Immobilization of Bacterial Isolates for Enhanced Phenol Degradation Efficiency

Production of Inoculum: To prepare bacterial isolates for immobilization, they were first inoculated in sterile nutrient broth and incubated for 48 hours at 30°C with shaking at 200 rpm. After incubation, the bacterial cells were harvested and immobilized within a gel matrix to restrict their mobility in the medium. In this study, the bacteria were trapped in calcium alginate beads.

Preparation of Embedding Medium: The embedding medium, or alginic acid gel, was prepared by mixing 2 g of sodium alginate with 50 ml of de-ionized water to create a 4% solution. This mixture was stirred carefully to avoid air bubbles, autoclaved at 121°C for 20 minutes, and then stored in a refrigerator at 4°C.

Preparation of Cross-Linking Reagent: The cross-linking reagent consisted of a calcium chloride solution. In a sterilized conical flask, 11.1 g of $CaCl_2$ was dissolved in 500 ml of de-ionized water to make a 0.2 M solution. This solution was then autoclaved at 121°C for 20 minutes.

Preparation of Immobilized Cells: Bacterial cells from isolates A2 and A7, grown in LB medium at 30°C for 48 hours with shaking at 200 rpm, were harvested by centrifugation. For the immobilization process, the cell pellets (1.0 g wet weight for A2) were mixed with the 4% sodium alginate solution. The cell-alginate mixture was then extruded through a tube and injected into the 0.2 M calcium chloride solution using an electrostatic droplet generator. This process allowed the formation of alginate beads containing the immobilized bacterial cells.

2.9 Efficient Number of Calcium alginate Beads for Phenol Degradation

A varying number of beads (1 bead/ml, 2 beads/ml, 3 beads/ml and 4 beads/ml) of immobilized cells of A2, A7 and a consortium of A2 and A7 isolates were mixed with an initial concentration of 1000 ppm of phenol separately. Then the tubes were incubated under static conditions at 37◦C for 72 hrs. Aliquots were taken periodically every 24 hrs. to calculate the percentage of degradation. Samples were withdrawn into serum vials and supernatant obtained after centrifugation at 10,000 rpm for 10 min was run through a spectrophotometer at 510 nm (λ max for phenol) [20].

3. Results

3.1 Isolation and Identification of the Potential Phenol-Degrading Bacteria

Contaminated soil and wastewater were used as a potential source to isolate high-performance phenol-degrading microorganisms. Bacterial isolates were tested for their ability to phenol degradation. Results showed that isolates from A1 to A9 had different degradation percentages for (20, 50, 75, 100 and 150 ppm) phenol concentrations ranged between 2.6 % and 99.9%. A2 and A7 isolated strains illustrated the highest degradation percentage from 86% to 99.9% after 72 hrs. of incubation. Isolates A2, A7, were selected for further experiment.

Identification of isolates A2 and A7 was performed through sequencing of the 16S ribosomal RNA gene (~960 bp). The sequences were analyzed using BLASTn and multiple sequence alignment with CLUSTAL W. Phenotypic characteristics and phylogenetic analysis were employed to construct a phylogenetic tree using BioEdit. The results identified isolate A2 as *Stenotrophomonas sp.*, showing 99% similarity to *Stenotrophomonas maltophilia* strain MSL_3045 (KT719879.1). The

sequence of isolate A2 was deposited in the GenBank database under accession number MH078250 (Fig 1). Isolate A7 was identified as *Pseudomonas sp.* and deposited under accession number MH078251 (Fig 2). The sequences were compared and identified using BLAST and RDP. A phylogenetic tree was constructed based on 16S rRNA gene sequences, with multiple alignments performed using CLUSTAL-X. Evolutionary distances were calculated using the Tamura-Nei model, and the tree was constructed using the Neighbor-Joining method in MEGA4 software, with a bootstrap analysis of up to 1,000 iterations.

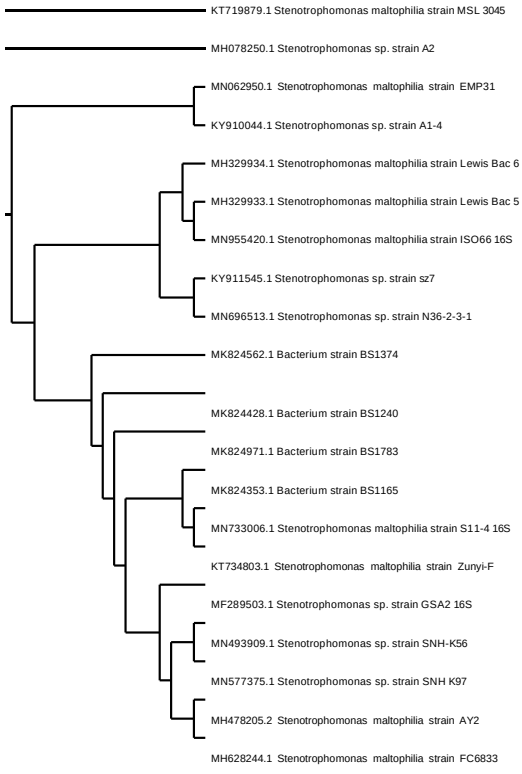

Figure 1. Phylogenetic tree illustrating the relationships between isolate A2 and its closest bacterial species. The tree was constructed using BioEdit software.

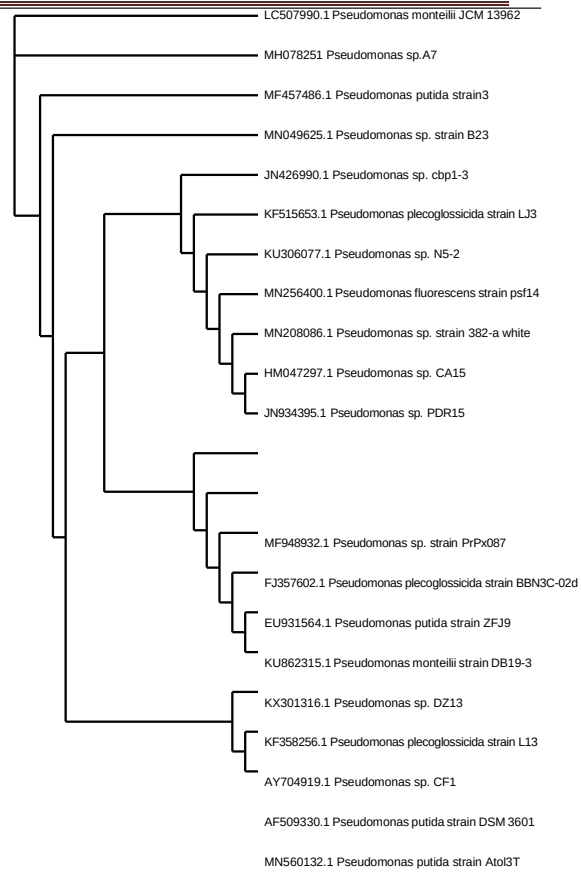

Figure 2. Phylogenetic tree illustrating the relationships between isolate A2 and its closest bacterial species. The tree was constructed using BioEdit software.

3.2 The Effect of Initial Phenol Concentration on its Degradation using Stenotrophomonas sp. A2 and Pseudomonas sp. A7.

Phenol concentrations degradation by the different strains was tested in the range from 100 mg/L to 1000 mg/L in 100 mg/L increments, as shown in Fig 3. The degradation percentage of phenol by *Stenotrophomonas* sp. decreased as the initial concentration increased, from 99.9% at 100 mg/L to 94.3% at 700 mg/L. Beyond 700 mg/L, the degradation further declined to 88% at 1000 mg/L. Interestingly, The degradation capacity of phenol by *Pseudomonas sp.* A7 reached over 95.4% up to 600 ppm. However, its efficiency decreased continuously with increasing phenol concentration, dropping to 86.3% degradation at 1000 ppm after 72 hours. This result indicates that isolated *Pseudomonas* sp strain maintains high degradation performance even at elevated initial phenol concentrations.

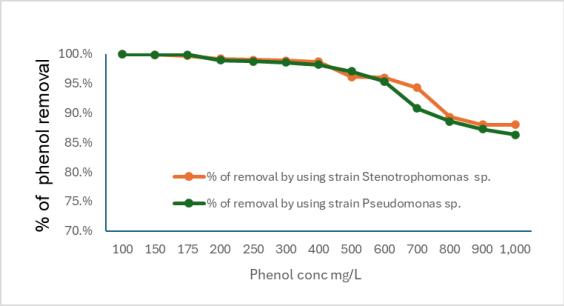

Figure 3. Influence of Phenol Concentration on Degradation Efficiency *by Stenotrophomonas sp*. and *Pseudomonas sp*.

3.3 Impact of Temperature Variation on Phenol Degradation Efficiency of Stenotrophomonas sp. and Pseudomonas sp.

The degradation efficiency of phenol by *Stenotrophomonas* sp. improved from 88.0% to 92.7% at an incubation temperature of 37°C under both static and shaking conditions after 72 hours. As the temperature increased from 20°C to 37°C, the degradation rate was consistent in both conditions. However, further increasing the temperature from 40°C to 50°C decreased the degradation percentage from 92.9% to 89%.

For *Pseudomonas sp*., the degradation efficiency improved from 86.0% to 89.7% at 37°C under shaking conditions and from 86.8% to 91.1% under static conditions, as the temperature increased from 20°C to 37°C. A further increase in temperature from 40°C to 50°C resulted in a 1% decrease in degradation efficiency.

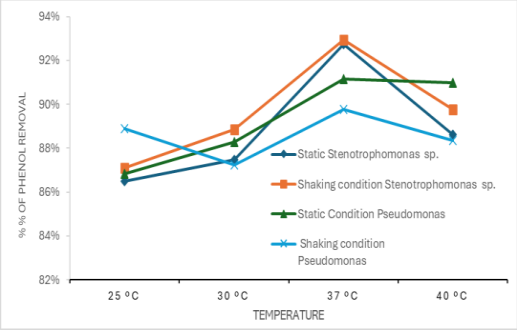

Figure 4. Impact of Temperature Variation on Phenol Degradation Efficiency of *Stenotrophomonas sp.* and *Pseudomonas sp.* under static and shaking conditions.

3.4 Influence of pH of Culture Medium on Phenol Degradation

The maximum phenol removal was observed at neutral pH. As shown in Figure (5), *Stenotrophomonas sp*. exhibited high degradation efficiency, achieving 92.8% and 92.1% at pH 6 and 7, respectively, after 72 hours of incubation with 1000 ppm of phenol under static conditions at 37°C. Phenol degradation decreased as the medium pH deviated from neutral, with pH levels above 7 showing a negative correlation due to inhibited growth, resulting in incomplete phenol degradation.

Similarly, Fig 5 demonstrates that *Pseudomonas sp*. showed high degradation efficiency, achieving 91.4% and 91.8% at pH 6 and 7, respectively, after 72 hours of incubation with 1000 ppm of phenol under static conditions at 37°C. As with *Stenotrophomonas sp*., phenol degradation decreased as the medium pH deviated from neutral, with pH levels above 7 negatively affecting growth and leading to incomplete degradation of phenol in the culture.

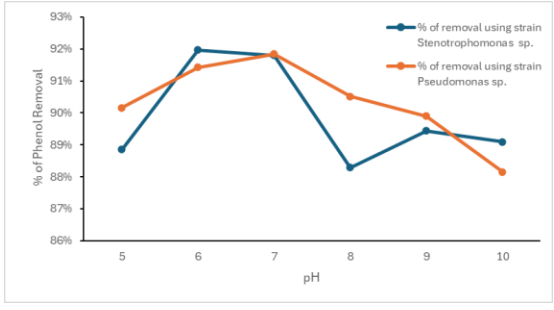

Figure 5. Impact of pH Variation on Phenol Degradation Efficiency of Stenotrophomonas sp. and *Pseudomonas sp.*

3.5 Impact of Contact Time Variation on Phenol Degradation Efficiency of Stenotrophomonas sp. and Pseudomonas sp

Stenotrophomonas sp.: The effect of contact time on phenol degradation by *Stenotrophomonas sp.* was studied at an initial phenol concentration of 1000 ppm, with samples withdrawn at 3-hour intervals under static conditions at 37°C. As shown in Fig 6, the degradation of phenol increased linearly with time. Complete degradation was achieved at the 24th hour, with a degradation efficiency of 92.16%.

Pseudomonas sp. A7: The impact of contact time on phenol degradation by *Pseudomonas sp*. A7 is also illustrated in Fig 6. Phenol was completely degraded within 24 hours, achieving a degradation efficiency of 91.76%.

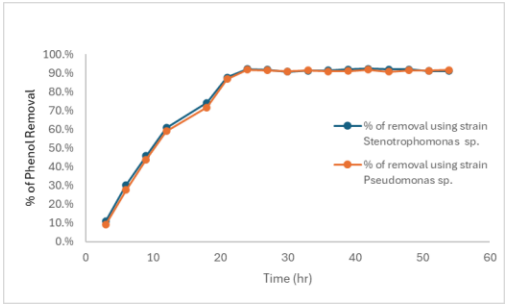

Figure 6. Impact of Contact Time Variation on Phenol Degradation Efficiency of *Stenotrophomonas* sp. and *Pseudomonas sp.*

3.6 Impact of Immobilization and Number of Beads on Phenol Degradation Efficiency of Stenotrophomonas sp. and Pseudomonas sp.

Stenotrophomonas sp.: The effect of the number of alginate beads on phenol degradation by *Stenotrophomonas sp*. was studied to determine the optimal bead count for maximum degradation (fig 7). Different numbers of beads (1, 2, 3, 4 beads/ml) were tested by adding them to 1000 ppm of phenol under optimal conditions (pH 7, 37°C, static) and measuring degradation after 72 hours. The degradation efficiency increased slightly with more beads, but the difference was not significant. Therefore, 2 beads/ml were used in subsequent experiments. The degradation percentage reached 93.0% with immobilized cells, compared to 92.56% with free cells under the same conditions.

Pseudomonas sp. A7: The degradation efficiency increased slightly with more beads, with no significant difference observed. Therefore, 2 beads/ml were used in further experiments. Immobilizing *Pseudomonas sp*. A7 in calcium alginate beads increased the degradation percentage to 92.0%, compared to 91.3% with free cells under the same conditions (Fig 7).

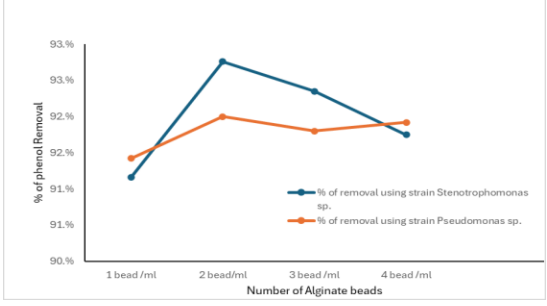

Figure 7. Impact of alginate beads number Variation on Phenol Degradation Efficiency of *Stenotrophomonas* sp. and *Pseudomonas sp*.

4. Discussion

Contaminated soil and wastewater were used for screening the biodegradation of phenol and its derivatives, the isolated bacteria were extensively studied, and a large number of phenol-degrading bacteria have been isolated and characterized at the physiological and genetic levels. Isolates A2, A7, were selected as the most potential phenol-degrading bacteria. The GenBank database was used to search for 16S rRNA sequences homologous to the 16S rRNA sequences of the new isolates.

Multiple alignments of different 16S rRNA gene sequences downloaded from GenBank were performed using CLUSTAL-X software. Evolutionary distances were calculated by the Tamura-Nei model. The phylogenetic tree was constructed by the Neighbor-Joining method by MEGA4 software (Tamura et al. 2007), and a bootstrap analysis of up to 1,000 iterations was carried out. Results identified the isolated strain A2 as *Stenotrophomonas* sp and A7 as *Pseudomonas* Sp.

Previous microbiological studies showed that pure and mixed cultures of the *Pseudomonas* genus are the most commonly utilized biomass for the biodegradation of phenols and they are believed to have good potential for different biotechnological applications [21]. Specifically, *Pseudomonas putida* has been commonly used for the biodegradation of phenol due to its high removal efficiency [22].

Microorganisms grow within a set range of physiological parameters, but maximum growth is achieved only at the optimum conditions of these physiological parameters. Different physiological parameters that usually interfere in the biodegradation activity of a microbe are incubation temperature, pH of the medium, and maximum toxicity of the phenol were studied [23].

The study on phenol degradation across varying initial concentrations provides insights into the performance of different bacterial strains. For *Stenotrophomonas sp*., the degradation efficiency declined as the phenol concentration increased, starting at 99.9% at 100 mg/L and decreasing to 94.3% at 700 mg/L. At concentrations above 700 mg/L, the degradation efficiency further dropped to 88% at 1000 mg/L. This trend indicates that while *Stenotrophomonas* sp. is highly effective at lower concentrations, its degradation capacity diminishes as the concentration of phenol increases. This could be attributed to the strain's potential limitations in handling higher loads of phenol, possibly due to the saturation of its metabolic pathways or enzyme systems.

In contrast, *Pseudomonas sp*. A7 demonstrated a high degradation efficiency of over 95.4% up to 600 mg/L. Although the efficiency decreased with rising concentrations, reaching 86.3% at 1000 mg/L, it still maintained relatively high performance compared to Stenotrophomonas sp.. This suggests that *Pseudomonas sp*. A7 has a robust degradation mechanism that allows it to effectively process phenol even at elevated concentrations. The continuous decrease in efficiency with higher concentrations reflects a similar challenge faced by many bacterial strains, where increased substrate concentration can inhibit degradation due to factors such as toxic effects or substrate inhibition.

Overall, these results highlight the superior phenol degradation capacity of *Pseudomonas sp*. A7 at higher concentrations compared to *Stenotrophomonas* sp., indicating its potential for more effective application in bioremediation processes involving high levels of phenol contamination.

Bioremediation studies showed the effect of contact time on phenol degradation by isolated P. putida. Indicating that phenol degradation occurred with the increase in the percentage degradation linearly with time. Phenol was completely degraded at 60 th hour. Polymenakou and Stephanou 2005 reported that, *Pseudomonas sp*. isolated from the petroleum-contaminated soil degrades phenol up to 1300 mg.l-1 within 156 hrs [24]. Similarly Chung, Tseng et al. 2003 also reported that, *Pseudomonas putida* degrades phenol up to 2000 mg.l-1 within 156 hrs [26]. Ravikumar, Parimala et al. 2011 reported that among thirty two morphologically different strains, *Pseudomonas putida*, *Bacillus* sp and *Pyrococcus horikoshii* was found as better strains for phenol degradation which are isolated from the retting water. In that Pseudomonas putida showed the complete degradation of phenol up to 2000 mg/L within 144 hrs [26].

Our results also demonstrate a clear, linear relationship between contact time and phenol degradation efficiency for the isolated *Stenotrophomonas sp.* The degradation efficiency increased progressively with time, culminating in complete degradation at the 24-hour mark, with a final degradation efficiency of 92.16%. This indicates that *Stenotrophomonas sp*. can effectively degrade phenol within a relatively short time frame under optimal conditions. The linear increase in degradation efficiency suggests a robust metabolic capacity of Stenotrophomonas sp. to utilize phenol as a carbon source, facilitating the breakdown of phenol consistently over time.

Similarly, *Pseudomonas sp.* A7 exhibited a high phenol degradation efficiency, achieving complete degradation within 24 hours with an efficiency of 91.76%. This result underscores the potential of *Pseudomonas sp*. A7 is a highly effective phenol-degrading bacterium. The efficiency observed in *Pseudomonas sp*. A7 was comparable to that of *Stenotrophomonas* sp., suggesting that both strains are well-suited for bioremediation applications involving phenol.

Temperature plays an important role than nutrient availability in the degradation of organic pollutants. Sudden exposure to temperatures higher than 35◦C may have a detrimental effect on the bacterial enzymes that are usually responsible for the benzene ring cleavage, which is the key step in the biodegradation process. On the other hand, exposure to temperatures much lower than 30◦C was expected to slow down the bacterial activity and enhance the inhibitory effect of phenol on the bacteria, especially for high phenol concentrations. Most of the studies on phenol biodegradation had been carried out in the temperature range of 25-35◦C [27].

The study on phenol degradation by *Stenotrophomonas* sp. revealed that degradation efficiency increased from 88.0% to 92.7% at 37°C under both static and shaking conditions after 72 hours. This suggests that the strain performs optimally at this temperature. However, further increasing the temperature to 50°C led to a decrease in degradation efficiency, from 92.9% to 89%. This decline indicates that higher temperatures may negatively impact the strain's performance.

Similarly, *Pseudomonas sp*. showed improved degradation efficiency, rising from 86.0% to 89.7% under shaking conditions and from 86.8% to 91.1% under static conditions at 37°C. Like *Stenotrophomonas* sp., an increase in temperature from 40°C to 50°C resulted in a slight decrease in degradation efficiency by 1%. These results underscore the importance of maintaining an optimal temperature for effective phenol degradation This is indifferent with previous data on *Pseudomonas* experiment shows P. desmolyticum degraded more phenol per day at 32° C than at any other temperature suggesting the value of both of the isolated trains exhibiting slightly reduced performance at higher temperatures.

Most of organisms cannot tolerate pH values below 4.0 and above 9.0 as it affects the metabolic pathway and denatures the proteins finally proving to be lethal [28].

For the isolated *Stenotrophomonas sp*., our results indicate high degradation efficiency at pH 6 and 7, with phenol removal efficiencies of 92.8% and 92.1%, respectively, after 72 hours of incubation at 1000 ppm phenol concentration under static conditions at 37°C. This suggests that

Stenotrophomonas sp. thrives and performs effectively in slightly acidic to neutral pH conditions. The decline in degradation efficiency at pH levels above 7 suggests that *Stenotrophomonas sp*. is sensitive to alkaline conditions, which negatively affect its growth and enzymatic activity, leading to incomplete phenol degradation.

Similarly, the isolated *Pseudomonas sp*. also demonstrated high degradation efficiency at pH 6 and 7, achieving 91.4% and 91.8% degradation, respectively, under the same conditions. The observed decrease in degradation efficiency at pH levels above 7 mirrors the results seen with *Stenotrophomonas* sp., indicating that *Pseudomonas sp.* also experiences reduced performance in alkaline environments. The pH-induced inhibition of growth and metabolic activity in both strains suggests that the phenol degradation processes are optimized in neutral to slightly acidic conditions.

These findings underline the importance of maintaining neutral pH conditions for maximizing phenol degradation in bioremediation applications. Both bacterial strains demonstrated optimal phenol degradation at pH levels close to neutral, reflecting their adaptation to environments with moderate acidity or alkalinity. The decreased efficiency observed at higher pH levels highlights the need to carefully control the pH in bioremediation systems to ensure effective degradation of phenolic compounds.

Previous research has shown that phenols tend to exist in undissociated form under these conditions and electrostatic force cannot prevent them from entering cells. Previous results indicated that the optimum for phenol degradation is 7.0 for *Pseudomonas putida* [29]. The follow-up of the medium pH can be an indicator of phenol degradation and one of the factors significant in the success of the biological treatment. The pH significantly affects the biochemical reactions required for phenol degradation. The rate of degradation also declines slightly for pH values higher than 7 and then stabilizes as reaches pH of 9 [30].

One of the key points for bioremediation is maintaining a high biomass of bacterial populations. To improve the survival and retention of the bioremediation agents in the contaminated sites, bacterial cells must be immobilized. Immobilized cells have been extensively used in the production of useful chemicals, treatment of wastewater and bioremediation of pollution cause of their longer operating lifetime and enhanced stability and survival of the cells [31]. To improve the survival and retention of the bioremediation agents in the contaminated sites, bacterial cells must be immobilized. Immobilized cells are widely tested for a variety of applications. Many types of support and immobilization techniques can be selected based on the sort of application [32].

The use of immobilized cells has been investigated as an alternative technology for environmental applications. These biocatalysts can offer the possibility of a wider and more economical exploitation in industry, waste treatment, medicine, development of bioprocess and monitoring devices like the biosensor

Stenotrophomonas sp. achieved a degradation efficiency of 93.0% with immobilized cells, compared to 92.56% with free cells. This suggests that while immobilization provides a modest improvement, the effect is relatively small, indicating that other factors may play a more significant role in enhancing degradation efficiency.

Similarly, for *Pseudomonas sp*. A7, increasing the number of beads also led to a slight improvement in degradation efficiency, with 2 beads/mL being chosen for subsequent experiments due to the lack of significant difference at higher concentrations. Immobilizing *Pseudomonas sp*. A7 in calcium alginate beads resulted in a degradation efficiency of 92.0%, compared to 91.3% with free cells. This shows a slight advantage of immobilization, although, like with *Stenotrophomonas sp*., the improvement is not dramatic.

Overall, while immobilization with alginate beads provides some benefit to phenol degradation for both bacterial strains, the improvement is modest. The choice of 2 beads/mL as the optimal concentration balances practicality and performance. The results suggest that while immobilization can enhance bacterial activity, the overall impact may be limited by factors such as bead permeability, bacterial attachment, and interaction with the phenol substrate. Further optimization of bead formulation and concentration may be needed to achieve more substantial improvements in degradation efficiency.

5. Conclusion

This study has successfully isolated and characterized phenol-degrading bacteria from contaminated soil and wastewater, identifying *Stenotrophomonas sp.* (A2) and *Pseudomonas sp*. A7 as highly effective strains for phenol bioremediation. Comprehensive physiological and genetic analyses, including 16S rRNA sequence comparisons using GenBank, confirmed the identities of these isolates. The phylogenetic analysis revealed that *Stenotrophomonas sp*. and *Pseudomonas sp*. A7 are well-suited for phenol degradation due to their efficient metabolic

pathways.

Our investigation into phenol degradation at various concentrations demonstrated that *Stenotrophomonas sp*. exhibited high degradation efficiency at lower concentrations but showed a decline as phenol concentration increased, from 99.9% at 100 mg/L to 88% at 1000 mg/L. In contrast, *Pseudomonas sp*. A7 maintained superior degradation performance at higher concentrations, with efficiencies exceeding 95.4% up to 600 mg/L and dropping to 86.3% at 1000 mg/L, highlighting its robustness in handling elevated phenol levels.

The impact of physiological parameters on degradation was also significant. Both strains showed optimal phenol degradation at neutral pH, with performance diminishing in alkaline conditions. Temperature studies revealed that both strains functioned most effectively at 37°C, with decreased efficiency at higher temperatures, underscoring the importance of maintaining optimal environmental conditions for effective bioremediation.

The study also demonstrated that immobilizing bacterial cells in alginate beads offered only modest improvements in degradation efficiency. *Stenotrophomonas sp*. and *Pseudomonas sp*. A7 showed slight enhancements in phenol degradation with immobilization, suggesting that while bead immobilization can be beneficial, it may not dramatically alter degradation performance. Further research into optimizing bead formulations and concentrations could provide more significant improvements.

Overall, *Pseudomonas sp*. A7 emerged as a particularly promising candidate for bioremediation of phenolic pollutants due to its high degradation capacity across a range of concentrations, making it a strong candidate for applications in environments with high phenol contamination.

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