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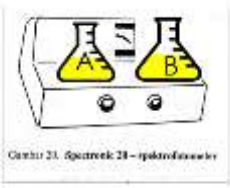
Degradation of Phenolic Compounds Using Pseudomonas aeruginosa and Enterobacter cloacae Bacteria in Groundwater (Case Study: Gempolsari Village)

Aulia Nur Febrianti[1], Dwi Ramadya Risqiana Putri[2], Rhenny Ratnawati[3]
[1,3]Department of Environmental Engineering, Faculty of Science and Technology, UIN Sunan Ampel Surabaya
[2]Department of Marine Science, Faculty of Science and Technology, UIN Sunan Ampel Surabaya

*Corresponding author
aulia.febrianti@uinsa.ac.id

Graphical Abstract



Abstract

The Lapindo mudflow disaster in Porong, Sidoarjo, has caused long-term environmental contamination, particularly affecting groundwater quality in surrounding residential areas such as Gempolsari Village. One of the major pollutants identified in contaminated groundwater is phenol, an aromatic hydrocarbon compound that poses significant risks to human health and aquatic ecosystems. This study aimed to evaluate the effectiveness of Pseudomonas aeruginosa and Enterobacter cloacae in degrading phenol compounds in groundwater contaminated by Lapindo mudflow under aerobic conditions. The biodegradation experiment was conducted using three treatment variations: variation A (Pseudomonas aeruginosa : Enterobacter cloacae = 10:0), variation B (5:5), and variation C (0:10). The initial phenol concentration in groundwater was 0.0168 ppm. Prior to treatment, groundwater quality parameters including pH, temperature, dissolved oxygen, and phenol concentration were analyzed. Observations of bacterial growth, pH changes, and phenol degradation were carried out at 6, 12, and 18 hours. The results showed that all treatment variations effectively degraded phenol and achieved complete removal at the 18th hour. Variation C demonstrated the best performance during the intermediate observation period, reducing phenol concentration from 0.0089 ppm to 0.0046 ppm at the 12th hour with a degradation efficiency of approximately 72.6%. The pH remained stable within the range of 6–7, indicating favorable conditions for bacterial activity. These findings indicate that Pseudomonas aeruginosa and Enterobacter cloacae have strong potential for the bioremediation of phenol-contaminated groundwater affected by Lapindo mudflow.

Keywords: Phenol Biodegradation, Pseudomonas aeruginosa, Groundwater Contamination

1. INTRODUCTION

The Lapindo mudflow disaster in Porong, Sidoarjo, East Java, Indonesia, is one of the largest and longest-lasting mud volcano disasters in the world. Since the eruption first occurred in May 2006, hot mud has continuously erupted from the subsurface and inundated large areas surrounding Porong District. The mudflow is estimated to release tens of thousands of cubic meters of mud every day, with several reports indicating an average discharge ranging from 120,000 to 160,000 m^3/day depending on geological pressure conditions and seasonal variations [1]. The continuous discharge of mud containing various inorganic and organic contaminants has raised serious environmental concerns, particularly regarding soil and groundwater pollution in nearby residential areas such as Gempolsari Village.

The area surrounding the Lapindo mudflow is densely populated and consists of residential settlements, agricultural land, transportation infrastructure, and industrial activities. Thousands of residents were displaced due to the inundation of villages, destruction of homes, and disruption of economic activities [2]. The disaster caused significant socio-economic impacts, including loss of livelihoods, decline in agricultural productivity, reduced land value, and deterioration of public health conditions. In addition, prolonged environmental contamination has increased public concern regarding the safety of groundwater resources used for domestic purposes by communities living near the mudflow area.

One of the major environmental concerns associated with the Lapindo mudflow is the presence of hydrocarbon compounds. Hydrocarbons are organic compounds composed primarily of carbon and hydrogen atoms and are commonly found in petroleum-derived materials [3]. These compounds may originate from deep geological formations brought to the surface through mud eruption processes. Hydrocarbons are generally classified into several groups, including aliphatic hydrocarbons, aromatic hydrocarbons, polycyclic aromatic hydrocarbons (PAHs), and phenolic compounds [4], [5], [6]. Many of these substances are toxic, persistent, and potentially harmful to aquatic ecosystems and human health when released into the environment without proper treatment [7].

Among hydrocarbon-derived pollutants, phenol is considered one of the most hazardous contaminants in groundwater systems. Phenol is an aromatic organic compound that is highly soluble in water and can easily infiltrate groundwater aquifers [8]. Exposure to phenol-contaminated water may cause adverse health effects, including skin irritation, respiratory disorders, liver and kidney damage, and toxic effects on the nervous system. In residential areas, groundwater contaminated with phenol poses serious risks because groundwater is commonly used for drinking, cooking, bathing, and other domestic activities [9]. Furthermore, phenol exhibits toxicity toward aquatic organisms and can reduce water quality even at relatively low concentrations, making it an important parameter in environmental pollution studies [10].

Various technologies have been developed to remove or degrade phenol in contaminated water. Conventional treatment methods include adsorption using activated carbon, chemical oxidation, membrane filtration, coagulation-flocculation, and advanced oxidation processes [11]. Although these methods can effectively reduce phenol concentration, several limitations such as high operational costs, sludge generation, and secondary pollution remain significant challenges. Therefore, biological treatment methods have gained increasing attention because they are considered environmentally friendly, cost-effective, and capable of degrading organic pollutants into simpler and less toxic compounds [12].

Biodegradation using microorganisms has been widely reported as an effective approach for phenol removal from contaminated environments [13]. Several studies have demonstrated that bacteria, fungi, and mixed microbial consortia possess enzymatic systems capable of utilizing phenol as a carbon and energy source. The use of bacterial combinations or consortium systems has shown higher degradation efficiency compared to single bacterial cultures due to synergistic metabolic interactions among microorganisms. Previous research reported that mixed bacterial cultures were able to improve phenol degradation rates, increase pollutant tolerance, and enhance adaptability under fluctuating environmental conditions [14].

Previous studies have demonstrated that *Pseudomonas aeruginosa* and *Enterobacter cloacae* possess significant potential for phenol biodegradation due to their high resistance to toxic aromatic compounds and rapid metabolic adaptation under aerobic conditions. Research conducted by Wang et al., [15] reported that *Pseudomonas* species exhibited high phenol degradation efficiency because of their ability to produce oxygenase enzymes that accelerate the breakdown of aromatic ring structures into simpler compounds. In addition, a study by Mahiuddin et al., [16] explained that *Enterobacter cloacae* showed strong tolerance to phenolic wastewater and could enhance biodegradation performance through synergistic interactions in mixed bacterial cultures. The selection of *Pseudomonas aeruginosa* and *Enterobacter cloacae* in this study was therefore based on their previously reported effectiveness, adaptability, and compatibility in aerobic phenol biodegradation systems. Furthermore, recent environmental monitoring studies in the Lapindo mudflow area reported that groundwater quality in several residential locations around Porong, including Gempolsari Village, still showed indications of contamination by organic pollutants such as phenolic compounds, highlighting the urgency of developing efficient and environmentally friendly bioremediation methods for contaminated groundwater treatment.

Among phenol-degrading bacteria, Pseudomonas aeruginosa is one of the most extensively studied microorganisms because of its strong metabolic capability to degrade aromatic compounds, including phenol [17]. This bacterium produces specific enzymes such as phenol hydroxylase and catechol dioxygenase that play important roles in the phenol biodegradation pathway [18], [19]. In addition, Enterobacter cloacae has also been reported to possess the ability to degrade phenolic compounds under various environmental conditions [18],

[20]. Both bacteria demonstrate significant potential for application in bioremediation processes of phenol-contaminated groundwater. However, studies investigating the combined application of Pseudomonas aeruginosa and Enterobacter cloacae for phenol degradation, particularly in groundwater contaminated by Lapindo mudflow in the Gempolsari area, remain very limited. Therefore, this study aims to evaluate the potential of these bacterial combinations in degrading phenol contaminants in groundwater affected by the Lapindo mudflow disaster.

2. METHOD

The research was conducted using a laboratory-scale biodegradation method to evaluate the potential of Pseudomonas aeruginosa and Enterobacter cloacae in degrading phenol compounds in groundwater contaminated by Lapindo mudflow. Initially, 200 mL of Nutrient Broth medium was prepared in 1000 mL Erlenmeyer flasks as a bacterial growth medium for Pseudomonas aeruginosa and Enterobacter cloacae. Each bacterial species was cultivated in two separate Erlenmeyer flasks. Subsequently, the bacterial isolates were inoculated into the prepared media and incubated for 24 hours under controlled laboratory conditions to promote bacterial growth and adaptation.

After the incubation process, each bacterial culture was transferred into two 1500 mL Erlenmeyer flasks containing 950 mL of sterile physiological saline solution. The bacterial concentration was adjusted until it reached 10^9 cells/mL using a hemacytometer for cell counting. Groundwater samples contaminated by Lapindo mudflow were then subjected to biodegradation testing using the prepared bacterial cultures. Several water quality parameters were analyzed before and after the treatment process, including temperature, pH, phenol concentration, and dissolved oxygen (DO), to evaluate the effectiveness of the bacterial degradation process on groundwater quality improvement.

Prior to the biodegradation experiment, initial characterization of the groundwater samples was conducted to determine the baseline conditions before treatment. The analyzed parameters included pH, phenol concentration, temperature, and dissolved oxygen (DO). Measurement of these parameters was intended to identify the initial groundwater quality and the level of phenol contamination in the study area before the addition of bacterial inoculum and nutrient supplementation. The initial data obtained were subsequently used as reference values to evaluate the effectiveness of the biodegradation process using Pseudomonas aeruginosa and Enterobacter cloacae in improving groundwater quality contaminated by Lapindo mudflow.

Table 1. Laboratory Results

Parameter	Lab Results	Standart
Phenol	0.0168 mg/liter	0.001 mg/liter
pH	6	6 – 9
Dissolved Oxygen	4	6
Temperature	28 C°	-

Experimental Variation

After both bacterial cultures reached a concentration of 10^9 cells/mL, the bacteria were introduced into groundwater samples that had been supplemented with nitrogen (N) and phosphorus (P) nutrients according to the predetermined nutrient ratio for each treatment variation. The biodegradation process was carried out under aerobic conditions by providing continuous aeration throughout the experiment to maintain sufficient dissolved oxygen for bacterial metabolic activity. The experimental setup consisted of three treatment variations, namely: Treatment A containing 6.3 L of groundwater inoculated with Pseudomonas aeruginosa and Enterobacter cloacae at a ratio of 10:0, Treatment B containing 6.3 L of groundwater inoculated with both bacteria at a ratio of 5:5, and Treatment C containing 6.3 L of groundwater inoculated with Pseudomonas aeruginosa and Enterobacter cloacae at a ratio of 0:10. Previous studies have reported that aerobic phenol biodegradation by bacterial isolates commonly shows significant degradation activity within the first 6–24 hours of incubation due to rapid bacterial adaptation and exponential growth phases [14]. Therefore, observation intervals at the 6th, 12th, and 18th hours were selected to evaluate the early degradation dynamics and determine the optimum bacterial performance during short-term biodegradation processes.

Phenol degradation performance was evaluated by analyzing phenol concentrations at specific observation times, namely at the 6th, 12th, and 18th hours of the biodegradation process. The analysis was conducted to determine the effectiveness of each bacterial treatment variation in reducing phenol concentrations in groundwater contaminated by Lapindo mudflow. In addition, monitoring degradation trends over time was intended to identify the optimum bacterial composition and operational duration for phenol removal under aerobic biodegradation conditions.

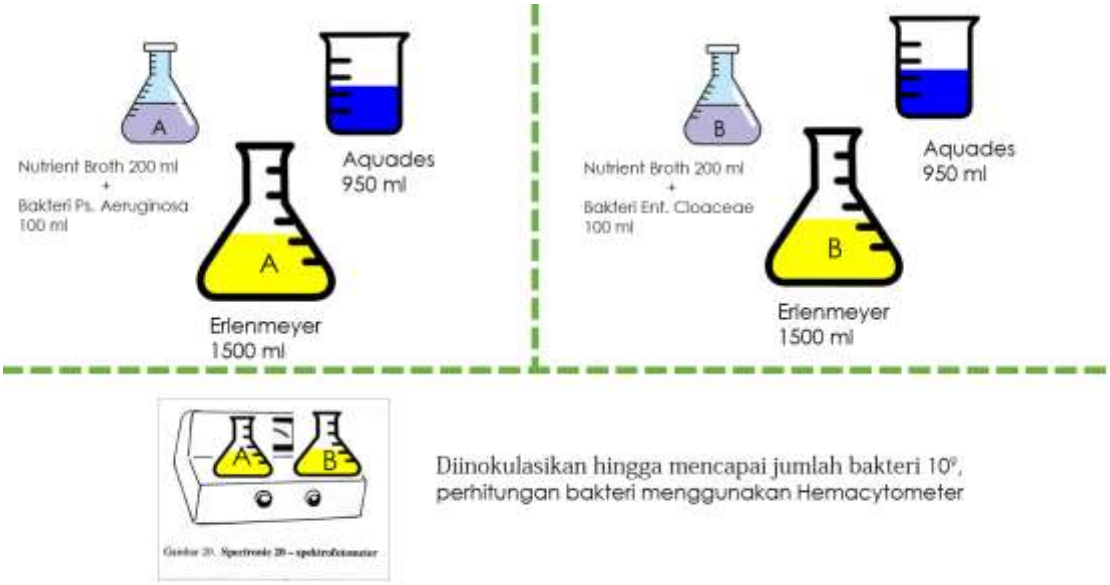


Figure 1. Bacterial Inoculation

3. RESULTS AND DISCUSSION

Effect of pH

Based on the table of pH changes in treatment variations A, B, and C, the pH values showed fluctuations during the observation periods of 6, 12, and 18 hours. In variation A, the pH changed from 6 at the 6th hour to 7 at the 12th hour, then decreased again to 6 at the 18th hour. Variation B showed a pH value of 7 at the 6th hour, which decreased to 6 at the 12th hour and remained stable until the 18th hour. Meanwhile, variation C had a pH value of 7 at the 6th hour, decreased to 6 at the 12th hour, and increased again to 7 at the 18th hour. These fluctuations indicate that bacterial activity during the biodegradation process influenced the chemical conditions of the groundwater over time.

The changes in pH during the biodegradation process were strongly influenced by the metabolic activities of Pseudomonas aeruginosa and Enterobacter cloacae in degrading organic compounds, particularly phenol. During phenol degradation, bacteria produce intermediate compounds such as simple organic acids that may temporarily lower the pH of the medium. However, at later stages, these acids can be further utilized by the bacteria as carbon sources, causing the pH to increase again toward neutral conditions [20], [21], [22]. This phenomenon explains the increase in pH observed in some treatment variations after specific incubation periods. In addition, continuous aeration contributed to maintaining pH stability by increasing dissolved oxygen levels, which supported aerobic biodegradation activity.

Pseudomonas aeruginosa is well known for its strong metabolic capability to degrade aromatic compounds such as phenol through enzymes including phenol hydroxylase and catechol dioxygenase, resulting in efficient biodegradation processes that influence pH changes in the medium [23], [24]. Meanwhile, Enterobacter cloacae is capable of adapting to various environmental conditions and producing metabolites during biodegradation that affect hydrogen ion balance in water. The combination of these two bacteria potentially creates synergistic metabolic interactions that cause dynamic pH fluctuations throughout the observation period. Overall, the pH values ranging from 6 to 7 indicate that the environmental conditions remained favorable for bacterial growth and phenol biodegradation during the experiment.

Table 2. Effect of pH

Variation	Time (Hours)		
	6	12	18
A	6	7	6
B	7	6	6
C	7	6	7

Number of Bacteria Growth

The graph illustrates the bacterial growth pattern observed in treatment variations A, B, and C during the biodegradation process at observation times of 6, 12, and 18 hours. In variation A, the number of bacteria increased significantly from approximately 1.2×10^6 cells at the 6th hour to nearly 1.0×10^7 cells at the 12th hour, and further increased to around 1.3×10^7 cells at the 18th hour. This trend indicates that the bacteria in variation A experienced a rapid exponential growth phase after an initial adaptation period. In variation B, the bacterial population slightly decreased from approximately 1.0×10^7 cells at the 6th hour to 8.3×10^6 cells at the 12th hour, before increasing again to approximately 1.1×10^7 cells at the 18th hour. Meanwhile, variation C showed a similar trend, where the bacterial population decreased from about 9.0×10^6 cells at the 6th hour to 7.1×10^6 cells at the 12th hour, then increased again to approximately 9.2×10^6 cells at the 18th hour.

The fluctuations in bacterial population observed in the graph are closely related to the bacterial growth phases during the biodegradation process. At the beginning of the experiment, bacteria entered the lag phase, in which microorganisms adapted to the environmental conditions, nutrient availability, and phenol concentration in the groundwater. During this phase, bacterial growth tends to be slow because cells are adjusting their metabolic activity and synthesizing enzymes required for phenol degradation[25]. After successful adaptation, the bacteria entered the exponential or log phase, characterized by rapid cell multiplication and increased biodegradation activity. The increase in bacterial population observed at the 18th hour indicates that the bacteria were actively utilizing phenol and other organic compounds as carbon and energy sources for growth [26].

The temporary decrease in bacterial numbers observed in variations B and C at the 12th hour may indicate environmental stress, substrate competition, or adaptation to phenol toxicity before the bacteria resumed active growth. Overall, the growth trends indicate that both Pseudomonas aeruginosa and Enterobacter cloacae were capable of surviving and proliferating during the biodegradation process, demonstrating their potential for phenol bioremediation in groundwater contaminated by Lapindo mudflow [27].

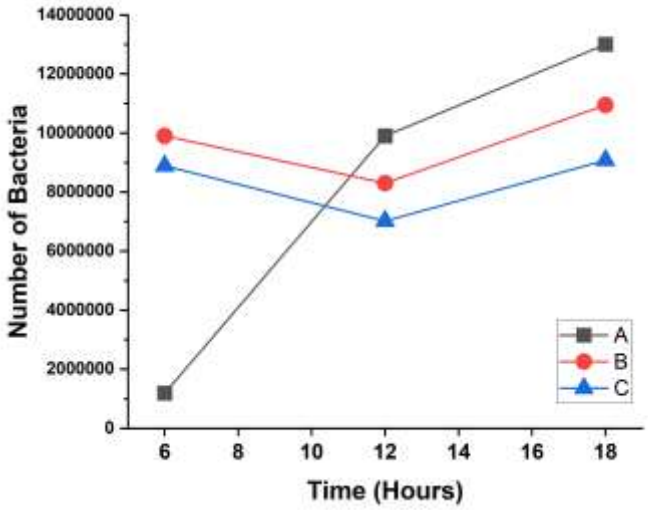


Figure 2. Bacterial Growth

Degradation of Phenol

The graph shows the reduction of phenol concentration during the biodegradation process at observation times of 6, 12, and 18 hours in treatment variations A, B, and C. In variation A, the phenol concentration decreased

from approximately 0.0095 ppm at the 6th hour to 0.0058 ppm at the 12th hour, and finally reached 0 ppm at the 18th hour. Variation B showed a decrease from 0.0080 ppm at the 6th hour to 0.0061 ppm at the 12th hour, before also reaching 0 ppm at the 18th hour. Meanwhile, variation C demonstrated the most rapid decrease, with phenol concentration declining from approximately 0.0089 ppm at the 6th hour to 0.0046 ppm at the 12th hour and reaching 0 ppm at the 18th hour. These results indicate that all bacterial variations were capable of degrading phenol effectively over time, with complete degradation observed after 18 hours of treatment.

The significant reduction in phenol concentration during the first 6 hours may be associated with the rapid adaptation phase of the bacteria under aerobic conditions. Recent studies reported that phenol-degrading bacteria such as Pseudomonas sp. and Enterobacter sp. are capable of initiating enzymatic oxidation shortly after inoculation when sufficient oxygen and nutrients are available. Research by Zhang et al. [28] showed that aerobic biodegradation systems could remove more than 50% of low-concentration phenol within the early incubation period due to high activity of phenol hydroxylase enzymes during the exponential growth phase. In addition, Kumar et al. [29] explained that continuous aeration enhances dissolved oxygen transfer, accelerates bacterial metabolism, and promotes faster cleavage of aromatic phenol structures into simpler compounds. Therefore, the drastic decrease observed between the initial concentration (0.0168 ppm) and the 6th-hour concentration indicates that the bacterial isolates rapidly adapted to the contaminated groundwater environment and efficiently utilized phenol as a carbon and energy source during the early stage of biodegradation.

The degradation of phenol occurred due to the metabolic activities of Pseudomonas aeruginosa and Enterobacter cloacae in utilizing phenol as a carbon and energy source. Pseudomonas aeruginosa possesses important enzymes such as phenol hydroxylase and catechol dioxygenase, which are involved in converting phenol into intermediate compounds that can be further metabolized through the β-ketoadipate pathway [30]. Similarly, Enterobacter cloacae has the ability to tolerate toxic organic compounds and biodegrade phenolic substances under aerobic conditions. During the biodegradation process, both bacteria break down the aromatic ring structure of phenol into simpler and less toxic compounds such as organic acids, carbon dioxide, and water. Continuous aeration also supported the degradation process by supplying dissolved oxygen required for aerobic bacterial metabolism [31], [32].

Based on the graph, variation C showed the best phenol degradation performance during the intermediate observation period because it produced the lowest phenol concentration at the 12th hour compared to variations A and B. If the initial phenol concentration was 0.0168 ppm, the degradation efficiencies at the 12th hour were approximately 65.5% for variation A, 63.7% for variation B, and 72.6% for variation C. At the 18th hour, all treatment variations achieved 100% phenol removal efficiency because the phenol concentration reached 0 ppm. However, variation C can be considered the most effective treatment because it demonstrated the fastest degradation rate and the highest phenol removal percentage during the earlier stages of the biodegradation process.

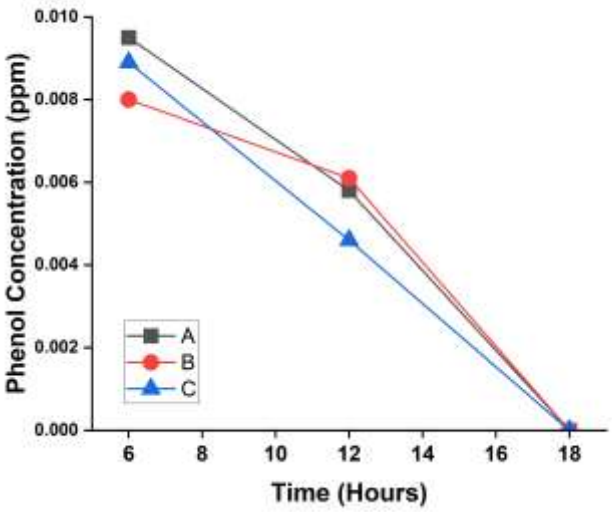


Figure 3. Phenol Degradation

4. CONCLUSION

The study results indicated that variation C was the most effective treatment for phenol biodegradation in groundwater contaminated by Lapindo mudflow. In this variation, the phenol concentration decreased more rapidly compared to variations A and B, dropping from approximately 0.0089 ppm at the 6th hour to 0.0046 ppm at the 12th hour, resulting in a degradation efficiency of around 72.6%. Although all treatment variations successfully achieved complete phenol removal at the 18th hour, variation C showed the highest degradation performance during the intermediate observation period. This suggests that the bacterial composition used in variation C was more efficient in accelerating the biodegradation process under aerobic conditions.

The superior performance of variation C was associated with the pH stability and bacterial growth dynamics observed throughout the experiment. The pH values remained within the range of 6–7, indicating environmental conditions that were suitable for the growth and metabolic activities of Pseudomonas aeruginosa and Enterobacter cloacae. Such pH conditions supported the enzymatic reactions involved in phenol degradation and maintained bacterial viability during the treatment process. Furthermore, even though a temporary reduction in bacterial population occurred at the 12th hour, the bacterial numbers increased again by the 18th hour, reflecting successful bacterial adaptation and continued biodegradation activity. This growth pattern demonstrated that both bacterial species were capable of utilizing phenol as a source of carbon and energy, thereby enhancing the overall efficiency of phenol removal from contaminated groundwater. From an operational perspective, the faster degradation performance observed in variation C also indicates potential advantages in terms of time and cost efficiency for large-scale bioremediation applications. Previous studies have reported that shorter biodegradation periods can reduce aeration energy consumption, operational time, and nutrient supplementation costs in aerobic bioremediation systems. Research conducted by Arutchelvan et al. [33] demonstrated that efficient phenol-degrading bacterial systems could significantly lower operational costs by minimizing hydraulic retention time while maintaining high removal efficiency. Similarly, Banerjee and Ghoshal [34] reported that rapid phenol degradation under aerobic conditions improves the economic feasibility of biological treatment systems due to reduced reactor operation duration and lower energy requirements. Therefore, the ability of variation C to achieve faster phenol degradation before the 18th hour suggests strong potential for more economical and practical implementation in groundwater remediation affected by Lapindo mudflow.

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