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Bioremediation of Phenol in Synthetic Tobacco Industry Liquid Waste Using Pseudomonas Putida

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ABSTRACT

Cigarette industry wastewater contains high phenol levels (100 mg/L), exceeding the regulatory limit of 0.5 mg/L (Minister of Environment Regulation No. 5/2014). Phenol is toxic, carcinogenic, and resistant to natural degradation, posing severe environmental and health risks. Existing treatments like ozonation (15.45% efficiency) and adsorption (60–80%) face limitations such as low efficiency and high operational costs. This research aimed to evaluate the bioremediation of synthetic tobacco wastewater using Pseudomonas putida, focusing on the effects of bacterial concentration (3–7%) and nutrient levels (0–2%) on phenol removal efficiency. A batch-culture experiment was conducted under controlled conditions (30°C, 150 rpm, 120 hours). Phenol concentration was measured via UV-Vis spectrophotometry, and bacterial growth was monitored using OD600. Data were analyzed using one-way ANOVA. The highest phenol removal (68%) occurred at 5% bacterial concentration and 2% nutrients, with OD600 = 1.000, indicating optimal bacterial growth. Nutrient variations did not significantly affect efficiency, but temperature fluctuations and carbon catabolite repression were identified as potential limiting factors. Pseudomonas putida offers a sustainable solution for phenol-laden wastewater, achieving near-compliance with regulatory standards. Future studies should optimize environmental controls and scale up the process for industrial applications.

Keywords: Biodegradation; Phenol; Pseudomonas putida; Synthetic wastewater; Tobacco.

INTRODUCTION

The cigarette production process includes three parts, namely *tobacco* processing, *clove* processing, and mixing (Yuliastuti & Cahyono, 2016). The liquid waste produced includes *tobacco* slurry, solvents, oils, and fats derived from the production process (Alabi et al., 2014). The *clove* processing process in the cigarette industry produces a phenol level of 100 mg/L (Yuliastuti & Cahyono, 2016). Phenol is a pollutant that is carcinogenic (Prayitno, 2016), difficult to degrade naturally, and stable in water, causing phenol content to be difficult to remove (Igwegbe et al., 2019). In accordance with the Regulation of the Minister of Environment No. 5 of 2014 concerning Wastewater Quality Standards, the maximum allowable level of phenol in the wastewater of cigarette/cigar factories is 0.5 mg/L. Therefore, further treatment is needed to reduce phenol levels.

There are several techniques for eliminating the phenol parameter in wastewater. Ozonization is one method for eliminating phenol (Asmura et al., 2017). However, even with a long exposure time, the efficiency of phenol elimination only reaches 15.45% (Mubarok, 2018). In addition to the ozonization method, there is the adsorption method, which is able to remove phenol in the range of 60–80% (Arif, 2014; Asyipa et al., 2021). However, adsorption requires a regeneration process or adsorbent washing after each use (Kuncoro & Soedjono,

2022). Besides the methods mentioned above, bioremediation is another approach that has the potential to remove phenol from wastewater.

Bioremediation is a remediation technique that uses bacteria, algae, or fungi to degrade organic and inorganic pollutants (Maulana & Mursiti, 2017). Bacteria that act as bioremediators can use pollutants as a source of protein to increase their biomass (Pratiwi et al., 2019). Environmental factors such as substrates (types and kinds of degraded compounds), temperature, and humidity affect the function of microorganisms (Melati, 2020).

Certain bacteria can be used to degrade aromatic compounds, including *Pseudomonas*, *Bacillus*, and *Nocardia* (Syafrizal, 2020). Moghadam et al. (2016) showed that the use of *Rhodococcus pyridinivorans* was able to degrade phenol at levels of 250, 500, and 750 mg/L. According to the research by Almajali et al. (2021), the use of *Curtobacterium flaccumfaciens* can also effectively reduce phenol levels by 700 ppm with an incubation time of 96 hours. In the study by Ereqat et al. (2018), the bacterium *Bacillus thuringiensis* was referenced for lowering the phenol level in the wastewater from olive oil factories (Farhadian et al., 2016; Ahmed et al., 2015). This bacterium achieved a phenol reduction efficiency of 88% with an incubation time of 96 hours.

Pseudomonas is a diverse and ecologically significant genus of bacteria, found both in terrestrial and marine environments. *Pseudomonas* is used in various biological processes due to its adaptability and ability to multiply in different environments (Syafrizal, 2020). In Mohanty's (2017) research, it was explained that properly acclimatized *Pseudomonas putida* can reduce phenol levels up to 1000 mg/L. Based on research by Salem et al. (2021), *Pseudomonas* sp. can degrade phenol by 1000 mg/L and shows resistance to phenol up to 1500 mg/L. According to Fatimah (2017), *Pseudomonas putida* is optimally used for phenol degradation at a concentration of 100 mg/L within 120 hours, achieving a reduction of over 80%. Therefore, this study pays particular attention to the effect of using *Pseudomonas putida* bacteria in reducing phenol levels in cigarette wastewater (Basak et al., 2021; Hasnain & Ahmed, 2023; Chen & Wang, 2017). The purpose of this study is to analyze the bioremediation process of synthetic liquid waste from the cigarette industry using *Pseudomonas putida*, with research modifications in the form of variations in bacterial and nutrient concentrations (Annuar et al., 2020; Gao et al., 2018; Khan & Ahmad, 2020).

This study aims to analyze the effectiveness of *Pseudomonas putida* in bioremediating phenol in synthetic tobacco industry wastewater, with variations in bacterial and nutrient concentrations (Chaudhary et al., 2019; Heinaru et al., 2019). The findings are expected to provide a sustainable and cost-effective solution for phenol removal, reducing environmental toxicity and improving wastewater treatment processes in the tobacco industry. Additionally, the research contributes to the broader field of bioremediation by optimizing conditions for bacterial degradation of phenol, which can be applied to other industrial waste streams.

METHOD

This study is a laboratory experimental study designed to evaluate the effectiveness of *Pseudomonas putida* in reducing phenol concentrations in synthetic liquid waste from the cigarette industry. The research design used a batch culture approach, with variations in bacterial and nutrient concentrations, to observe their effect on phenol removal efficiency under controlled conditions.

Materials and Tools

The main ingredients used include Pseudomonas putida bacterial cultures (pure strains), technical phenols, additional nutrients (such as glucose), and sterile aquadest solutions. The equipment used in this study includes UV-Vis spectrophotometers, shaker incubators, autoclaves, pH meters, Erlenmeyer flasks, and other laboratory glassware.

Experimental Procedure

a) Preparation of Bacterial Culture

Pseudomonas putida culture is activated in nutrient broth (NB) media and incubated at a temperature of 30 °C for 24 hours. The bacterial suspension is then adjusted in concentration to achieve 3%, 4%, 5%, 6%, and 7% (v/v) variations.

b) Synthetic Liquid Waste

Synthetic liquid waste is prepared by dissolving phenols in sterile aquadest until an initial concentration of 100 mg/L is reached. Nutrients are added according to treatment variations (0%, 0.5%, 1%, and 2%).

c) Bioremediation Initiation

The phenol, nutrient, and bacterial culture solution are placed into a sterile Erlenmeyer flask (capacity 250 mL) and incubated in a shaker at 30 °C and a speed of 150 rpm for 120 hours. Sampling is conducted every 24 hours.

d) Parameter Analysis

The phenol concentration is analyzed using UV-Vis spectrophotometry at the maximum wavelength of the phenol. Bacterial biomass growth is measured based on the Optical Density value at a wavelength of 600 nm (OD_{600}).

Data Analysis

The efficiency of the allowance is calculated using an equation that refers to the research of Romadhonah (2021), % *Allowance* = $(A-B) / A \times 100\%$

Information:

A: *Initial Concentration* (mg/L)

B: *Final Concentration* (mg/L)

Data were analyzed descriptively and statistically using variance analysis (one way ANOVA) to evaluate the effect of treatment variation on biodegradation efficiency.

RESULTS AND DISCUSSION

Characteristics of Cigarette Industry Wastewater

The characteristics of the wastewater of the cigarette industry in this study were obtained through the manufacture of synthetic wastewater referring to the journal owned by Hao et al. (2022) with selected parameters, namely phenol and pH. The initial concentrations of phenols as well as phenols can be seen in Table 4.1.

Table 1. Characteristics of Cigarette Industry Wastewater *)

No.	Parameter	Unit	Concentration Beginning	Baku Mutu*
1	Fenol	mg/L	100	0,5
2	pH	-	7	6-9

Source: Researcher synthesis data, 2024

The determination of the initial concentration of phenol parameters refers to the journal Yuliastuti & Cahyono (2016) explaining that the range of phenol levels in cigarette industry wastewater is 100 mg/L before treatment. The initial pH of synthetic wastewater was stabilized at 7 based on previous research showing that Pseudomonas putida bacteria experienced optimal growth and could degrade optimally when the pH level of wastewater was at 7 or neutral (Fatimah, 2017; Nguyen et al., 2023).

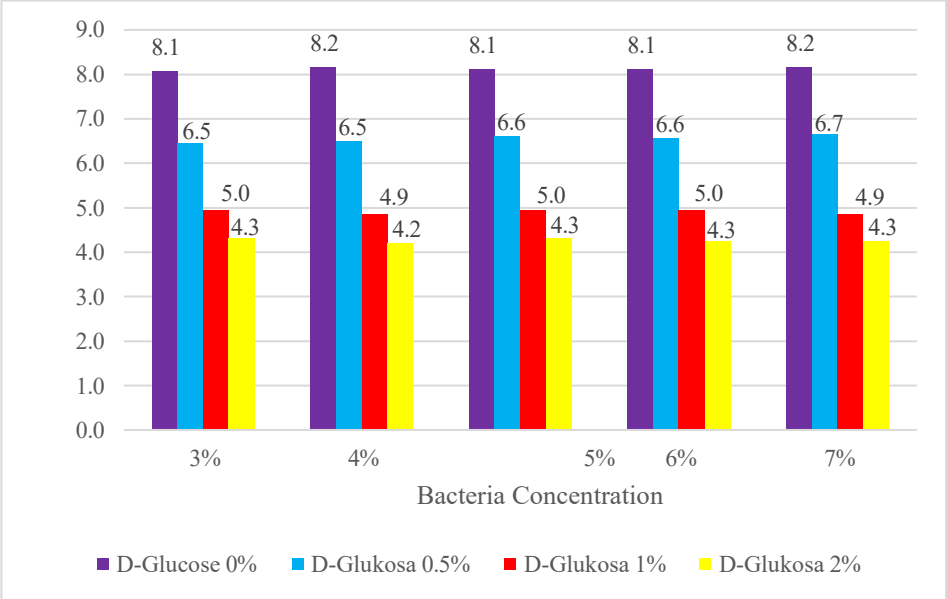

Figure 1. pH level on Day 5 (120 hours)

Source: Experimental results (Researcher, 2024)

There are changes that occur in the pH of wastewater after the addition of bacteria and nutrients. There was an increase in the pH of the wastewater in the control reactor (7-8.2), while in the reactor where nutrients were added there was a decrease in the pH of the wastewater as can be seen in Figure 4.1. Basically, Pseudomonas putida bacteria are aerobic bacteria that have alkaline by-products, but the addition of nutrients in the form of D-glucose can trigger the formation of citric acid, pyruvic acid and acetate acid in D-glucose metabolism resulting in a decrease in wastewater pH (Mahin et al., 2011).

Analysis of Phenol Elimination Efficiency by Variations in Bacterial Concentrations

The effect of the concentration of Pseudomonas putida bacteria on the efficiency of phenol parameter elimination is shown in Figure 2, Figure 3, Figure 4 and Figure 5. The similarity between Figure 2, Figure 3, Figure 4 and Figure 5 is the increase in the efficiency of the dispensing between the 24th and 48th hours, this is due to the condition of bacteria entering an exponential phase or rapid growth (Risna et al., 2022).

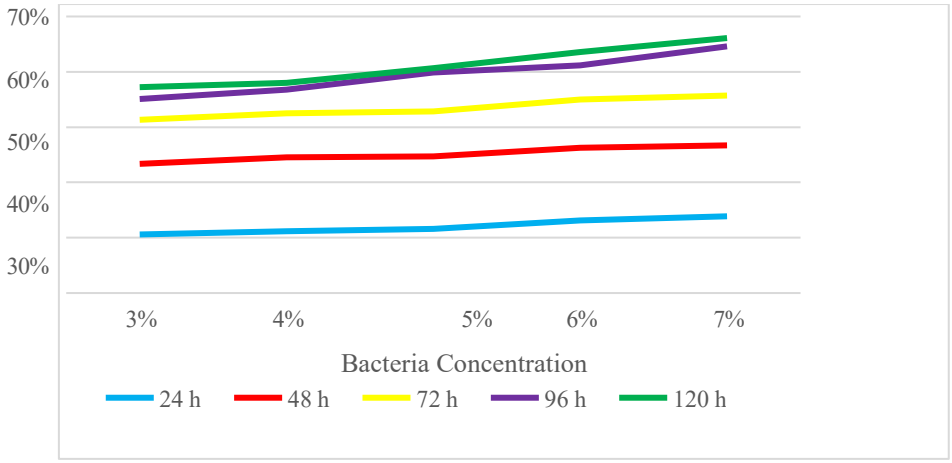

Figure 2. Effect of Variations in Bacterial Concentration on Phenol Elimination in Control Circumstances

Source: Experimental results (Researcher, 2024)

In Figure 2, it can be seen that under control conditions or without the addition of nutrients, the more bacteria concentration is added, the higher the efficiency of phenol removal by Pseudomonas putida bacteria. In addition, the longer the stay time given, the higher the phenol allowance will also be (Fatimah, 2017). Therefore, it can be seen that the optimum bacterial concentration is at 7% at the 120th hour with a phenol elimination efficiency of 66%.

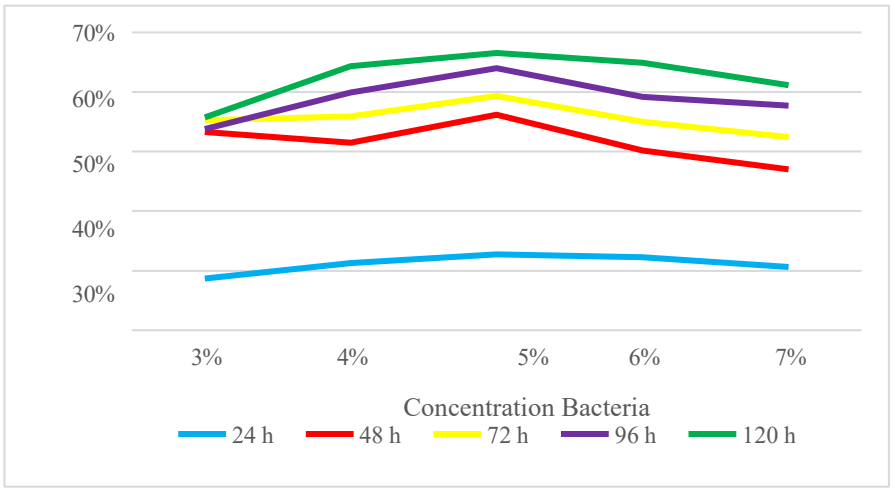

Figure 3. Effect of Variations in Bacterial Concentration on Phenol Elimination in the D-Glucose State 0.5%

In Figure 3, it can be seen that in the state of D-Glucose addition of 0.5%, the optimum condition is at a bacterial concentration of 5% with a stay time of 120 hours. This can be caused by the growth of high bacteria with an OD600 of 0.871 at the time of 5% bacteria concentration at the 120th hour, attached in Figure 6. It shows that the higher the growth of bacteria, the efficiency of the allowance will also increase (Lin, 2021; Mohanty & Jena, 2017).

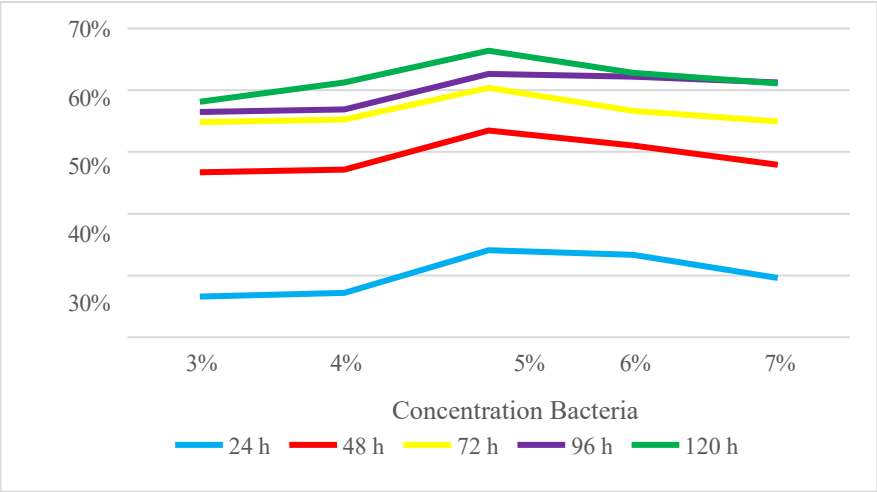

Figure 4. Effect of Variations in Bacterial Concentration on Phenol Elimination in the D-Glucose State 1%

Source: Experimental results (Researcher, 2024)

In Figure 4, it can be seen that the optimum conditions for elimination by Pseudomonas putida bacteria are at a concentration of 5% with a residence time of 120 hours. This can be caused by the growth of high bacteria with an OD600 of 0.983 at the time of 5% bacteria concentration at the 120th hour, attached in Figure 6. It shows that the higher the growth of bacteria, the efficiency of the allowance will also increase (Lin, 2021; Mohanty & Jena, 2017).

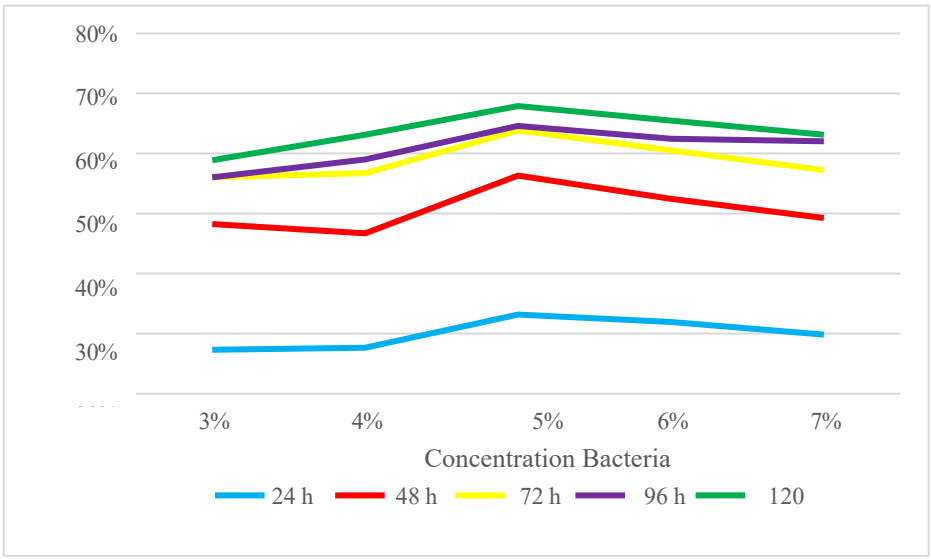

Figure 5. Effect of Variations in Bacterial Concentration on Phenol Elimination in the D-Glucose State 2%

Source: Results of this study (Researcher, 2024)

In Figure 4.5, it can be seen that the optimum elimination of phenols is at a residence time of 120 hours with the concentration of Pseudomonas putida bacteria at 5%. This can be caused by the growth of high bacteria at OD600 i.e. 1,000 at the time of 5% bacteria concentration at the 120th hour, attached to Figure 6. shows that the higher the growth of bacteria, the efficiency of the dispensing will also increase (Lin, 2021; Mohanty & Jena, 2017).

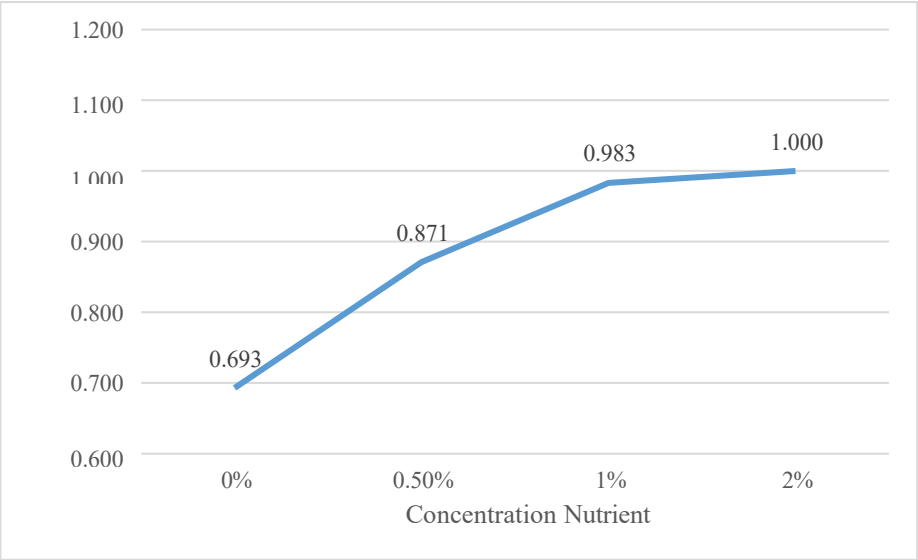

Figure 6. OD600 in Pseudomonas putida Bacterial Variation at 5%
Source: Measurement of OD600 in this study (Researcher, 2024)

Analysis of Phenol Elimination Efficiency by Variations in Bacterial Concentrations

The effect of nutrient concentration on the efficiency of phenol parameter elimination is shown in Figure 7, Figure 8, Figure 9, Figure 10 and Figure 11. There are similarities in Figure 7, Figure 8, Figure 9, Figure 10 and Figure 11, namely an increase in the efficiency of the preparation between the 24th and 48th hours, this is due to the condition of bacteria entering the exponential phase or rapid growth (Risna et al., 2022). Figure 7 Effect of Variation in Nutrient Concentration on Phenol Elimination in the Bacterial State of 3%

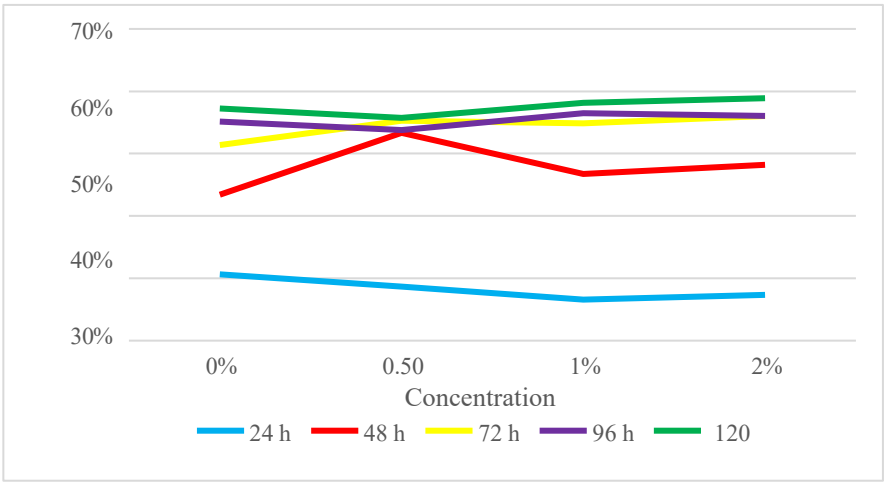

Figure 7. Effect of Variation in Nutrient Concentration on Phenol Elimination in the Bacterial State of 3%
Source: experimental data (Researcher, 2024)

In Figure 7, it can be seen that the optimal condition of phenol elimination is at a nutrient concentration of 2% during a 120-hour stay of 59% with an OD600 value of 0.822. In addition,

in Figure 7 there is an increase and decrease in efficiency when the concentration of nutrients is at 0.5% during the 96th to 120th hour range. The efficiency of the dispensing is closely related to the growth phase of bacteria, from the OD600 data obtained. The hourly increase is at a time when the concentration of nutrients is at 0.5%. The inverse comparison between OD600 and the efficiency of the dispensing can be due to the inappropriate room temperature (19-24°C) due to the absence of incubator use, whereas Pseudomonas putida bacteria are optimal in the range of 30-40°C (Munna et al., 2016). The impact of inappropriate temperatures can lead to inhibition of bacterial growth and decreased phenol elimination efficiency.

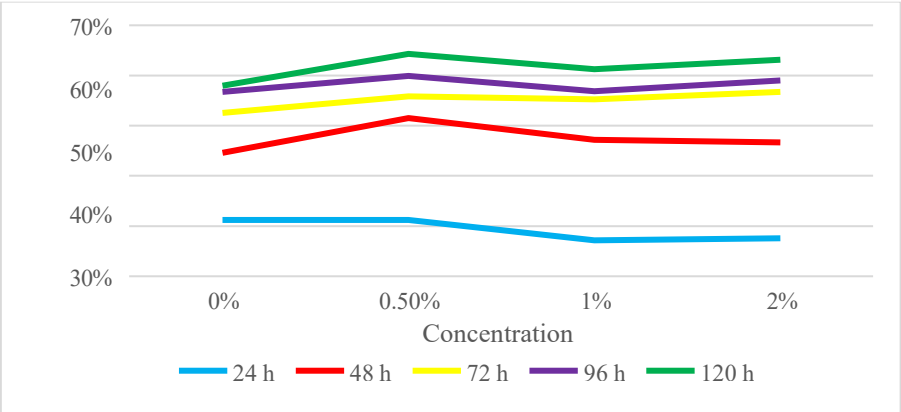

Figure 8. Effect of Nutrient Concentration Variation on Phenol Elimination in the Bacterial State of 4%.

Source: Results of this study (Researcher, 2024)

In Figure 8, it can be seen that the optimum condition of the preparation falls at the time of nutrient concentration of 0.5% and the residence time is 120 hours with an efficiency value of 64% and OD600 of 0.788.

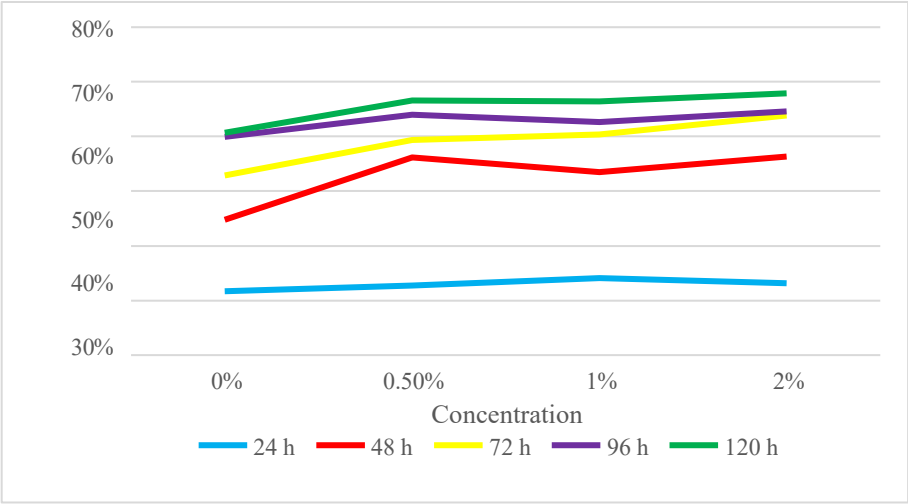

Figure 9. Effect of Nutrient Concentration Variation on Phenol Elimination in the Bacterial State 5%

Source: Experimental data (Researchers, 2024)

In Figure 9, it can be seen that the optimum conditions of the preparation fall at the time of nutrient concentration of 2% and the stay time of 120 hours with an efficiency value of 68%

and OD600 of 1,000. Figure 10 Effect of Variation in Nutrient Concentration on Phenol Elimination in the Bacterial State 6%.

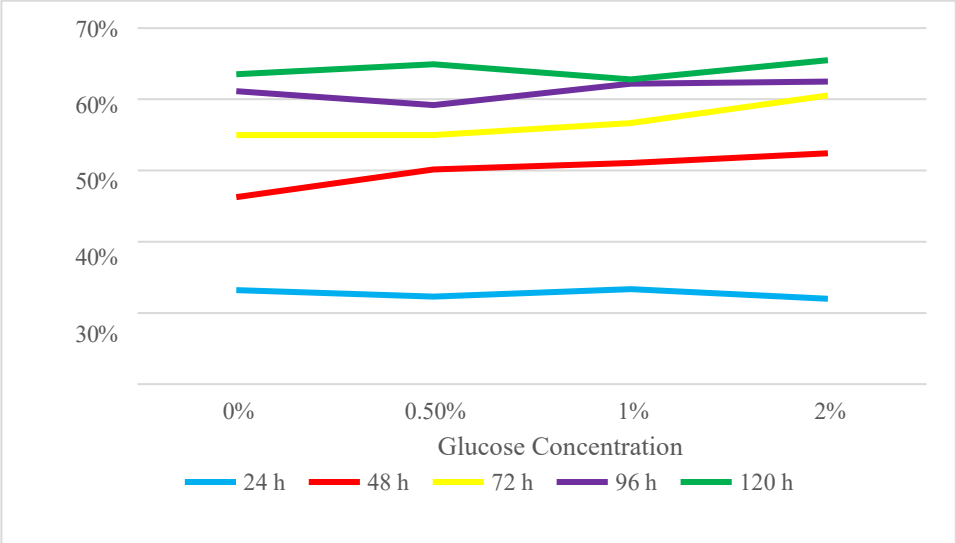

Figure 10. Effect of Variation in Nutrient Concentration on Phenol Elimination in the Bacterial State 6%

Source: Results of this study (Researcher, 2024)

In Figure 10, it can be seen that the optimal conditions for the preparation of the fall are at the time of nutrient concentration of 2% and the stay time is 120 hours with an efficiency value of 65% and OD600 of 0.956.

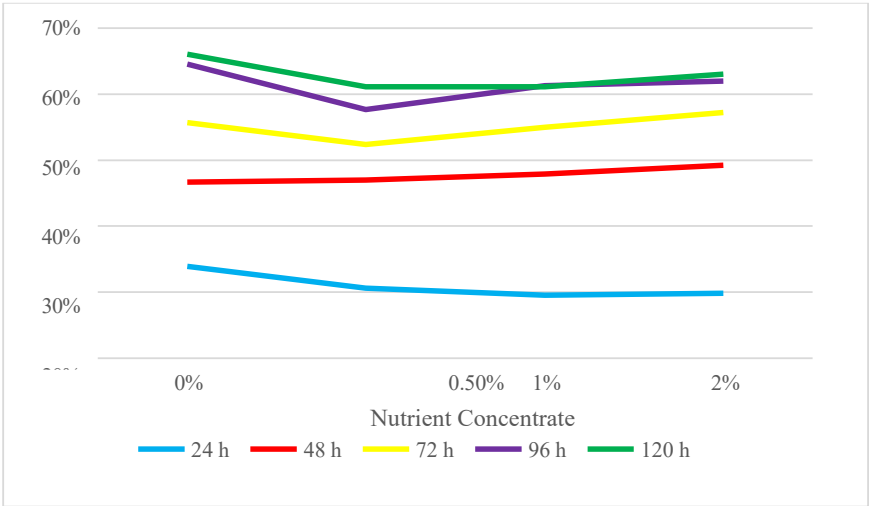

Figure 11. Effect of Nutrient Concentration Variation on Phenol Elimination in the Bacterial State 7%

Source: Experimental data (Researchers, 2024)

In Figure 11, it can be seen that the optimal conditions of the preparation fall at the time of 0% nutrient concentration and a stay time of 120 hours with an efficiency value of 65% and an OD600 of 0.763. From Figure 7, Figure 8, Figure 9, Figure 10 and Figure 11, the results of OD600 and inappropriate elimination efficiency were obtained, due to the decreased phenol elimination efficiency as the bacterial growth increased. According to research by Lin (2021) and Mohanty (2017), if the growth of bacteria increased, the efficiency of the allowance will

also increase. This can be caused by several reasons, including uncontrolled temperature and carbon catabolite repression (CCR). Temperature has an important role in the growth of a bacterium, basically for Pseudomonas putida bacteria will be in the optimal phase when the temperature is in the range of 30-40°C. If the temperature is not suitable, it can inhibit the growth of the bacteria (Munna et al., 2016). Another reason is CCR, which is a state in which bacteria prefer other carbon sources besides phenols, namely D-Glucose (Rojo, 2010). Therefore, this condition leads to a decrease in phenol elimination efficiency.

ANOVA One Way Statistic Analysis

Analysis of the Effect of Pseudomonas putida Concentration Variations on Phenol Elimination Efficiency In order to determine the effect of Pseudomonas putida concentration variations on phenol elimination efficiency, it was carried out using the SPPS program. First, a normality test was carried out on the data that had been obtained.

Table 2. Normality Test*)

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Bacteria	**Statistic**	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
Efficiency	Bacteria 3%	.151	4	.	.993	4	.972
	Bacteria 4%	.215	4	.	.946	4	.689
	Bacteria 5%	.314	4	.	.854	4	.240
	Bacteria 6%	.283	4	.	.863	4	.272
	Bacteria 7%	.271	4	.	.848	4	.220

Source: Statistical analysis using SPSS, 2024

In the normality test, there are two interpretations of the data if sig. > 0.05, the data obtained is spread normally. If the sig < 0.05, then the data obtained is not distributed normally and cannot be carried out the ANOVA One Way test. Table 4.3 shows that the distribution of the data is considered normal due to the sig. > 0.05. Hypothesis was made first before proceeding to the ANOVA test, namely: H0 : No significant difference in phenol elimination efficiency by variations in Pseudomonas putida concentration

H1 : There is a significant difference in phenol elimination efficiency by variations in Pseudomonas putida concentrations

If the results of the ANOVA test are obtained sig. > 0.05 then H0 is accepted and H1 is rejected, and vice versa, if the result is sig. < 0.05 then H1 is accepted and H0 is rejected. The results of the data analysis showed that the significance obtained was 0.001, so H0 was rejected and H1 was accepted. From the data obtained, it can be seen that the variation in the concentration of Pseudomonas putida bacteria has a significant effect on the elimination of phenol parameters.

Analysis of the Effect of Variation in Nutrient Concentration on Phenol Elimination Efficiency

In order to determine the effect of variations in nutrient concentration on phenol elimination efficiency, it was carried out using the SPPS program. First, a normality test is

carried out on the data that has been obtained, the results of the normality test can be seen in Table 3.

Table 3. Normality Test*)

		Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Glucose	**Statistic**	**df**	**Sig.**	**Statistic**	**df**	**Sig.**
Efficiency	Glucose 0	.198	5	.200*	.939	5	.658
	Glucose 0.5	.228	5	.200*	.936	5	.636
	Glucose 1	.207	5	.200*	.967	5	.853
	Glucose 2	.228	5	.200*	.967	5	.858

Source: Normality test results using SPSS, 2024

In the normality test, there are two interpretations of the data if sig. > 0.05, the data obtained is spread normally. If the sig < 0.05, then the data obtained is not distributed normally and cannot be carried out the ANOVA One Way test (Mortezaeikia et al., 2017). Table 5 shows that the distribution of the data is considered normal due to the magnitude of the data. > 0.05. Hypothesis was made first before proceeding to the ANOVA test, namely: H0 : No significant difference in phenol elimination efficiency by variation in nutrient concentration
H1 : There is a significant difference in phenol removal efficiency by variations in nutrient concentrations

If the results of the ANOVA test are obtained sig. > 0.05 then H0 is accepted and H1 is rejected, and vice versa, if the result is sig. < 0.05 then H1 is accepted and H0 is rejected. The results of the data analysis showed that the significance obtained > 0.05, so H1 was rejected and H0 was accepted (Salem et al., 2021). From the data obtained, it can be seen that the variation in nutrient concentration does not have a significant effect on the elimination of phenol parameters (Wang et al., 2022; Nguyen et al., 2023)..

CONCLUSION

Based on the results of the research obtained, it can be concluded that variations in the concentration of *Pseudomonas putida* bacteria have a significant effect on the elimination of phenol parameters. Meanwhile, variations in nutrient concentration did not have a significant effect on the elimination of phenol parameters. For further research development, it is recommended to optimize environmental conditions by using an incubator to maintain an optimal temperature (30–40 °C). In addition, it is necessary to explore alternative sources of nutrients to reduce the effects of *CCR*, as well as conduct trials on a larger scale or with real industrial waste to assess the feasibility of application. Combining this process with other treatment methods, such as *adsorption*, can also be considered to improve phenol removal efficiency. Long-term evaluation of the bacteria's performance is also necessary to ensure the sustainability of the bioremediation process. These measures are expected to provide an effective and environmentally friendly solution for the treatment of waste containing phenols.

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