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# EFFECTS OF *AZOTOBACTER VINELANDII* AND *PSEUDOMONAS SP.* INOCULATION ON THE REMOVAL OF PETROLEUM HYDROCARBONS DURING PHYTOREMEDIATION USING *SORGHUM BICOLOR L.*

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## ABSTRACT

Synergistic interactions between plants and bacteria can be utilized to enhance the performance of phytoremediation in hydrocarbon (HC)-contaminated soils. This study aimed to characterize plant growth–promoting rhizobacteria, Azotobacter vinelandii. and Pseudomonas sp., in improving the phytoremediation performance of petroleum waste using sorghum (Sorghum bicolor L.). The experiment employed a factorial randomized block design (RBD-factorial), consisting of Azotobacter vinelandii. inoculation at 0%, 1%, 2%, and 3% per total petroleum hydrocarbon (TPH) concentration, and Pseudomonas sp. inoculation at 0%, 1%, 2%, and 3% per TPH load. The results showed no interaction between Azotobacter vinelandii. and Pseudomonas sp. inoculation on all response variables. However, independent effects were observed on hydrocarbon biodegradation efficiency. Inoculation with Azotobacter vinelandii. significantly increased hydrocarbon (TPH) removal efficiency compared to the control at application rates of 1%, 2%, and 3% per TPH load during the 14th week after planting (WAP), with efficiencies of 68.160%, 76.656%, 74.260%, and 75.817%, respectively. Meanwhile, Pseudomonas sp. inoculation during the same period increased hydrocarbon biodegradation efficiency only at the 2% per TPH load treatment, with a value of 72.613%. Both inoculants functioned as biostimulants in enhancing petroleum hydrocarbon biodegradation efficiency; however, they did not contribute to increasing indigenous petrobacteria populations or sorghum plant height growth. Independently, Azotobacter vinelandii. and Pseudomonas sp. acted as biostimulants that stimulated the degradation of petroleum hydrocarbons.

**Keywords:** *Azotobacter vinelandii*., hydrocarbon, phytoremediation, Pseudomonas sp., Sorghum *bicolor L.*

## 1. INTRODUCTION

Petroleum contamination in soil is a major environmental problem that can significantly reduce soil productivity for agricultural purposes. The Central Statistics Agency of the Republic of Indonesia (BPS, 2020) reported that crude oil production in 2019 reached 259,246.8 thousand barrels, with approximately 37.38% of this amount generated as waste. Petroleum waste originates from various activities in the oil and gas industry, ranging from exploration to

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processing stages. The most dominant and regulated type of petroleum waste is that deposited at disposal sites or resulting from oil spill incidents. According to the Decree of the State Minister for the Environment No. 128 (2003), oil and gas industries are required to rehabilitate land contaminated by petroleum waste at disposal areas. This obligation aims to preserve environmental sustainability and to prevent the toxic effects of hydrocarbon compounds.

Petroleum, or crude oil, is composed primarily of carbon and hydrogen that have been deposited over long geological periods under high pressure and temperature conditions in fossil form. Under these conditions, carbon bonds are transformed into hydrocarbon chains (Munawar, 2012). The composition of petroleum consists of approximately 85% carbon, 12% hydrogen (H), 0–0.5% nitrogen (N), 0–6% phosphorus (P), 0–3.5% oxygen (O), sulfur (S), and 0–0.1% trace metals such as arsenic (As), cadmium (Cd), chromium (Cr), mercury (Hg), nickel (Ni), lead (Pb), copper (Cu), and zinc (Zn) (Suryatmana et al., 2007; Karwati, 2009).

Petroleum hydrocarbon compounds are classified into four main groups: (1) saturated hydrocarbons, including aliphatic and alicyclic alkanes; (2) aromatic hydrocarbons, consisting of monoaromatic and polycyclic aromatic hydrocarbons; (3) resins, which are polar compounds containing nitrogen, sulfur, and oxygen; and (4) asphaltenes, which are high-molecular-weight compounds associated with metals (Suryatmana, 2006; Munawar, 2012). Due to their persistence, toxicity, and carcinogenic properties, petroleum hydrocarbons are categorized as hazardous substances that require appropriate remediation measures.

One environmentally friendly technique for remediating petroleum-contaminated land is phytoremediation. The principle of phytoremediation is based on the ability of plants to release root exudates into the rhizosphere, which can increase the population of functional microorganisms by 5–100 times compared to non-rhizosphere zones, thereby enhancing hydrocarbon degradation (Melethia et al., 1996).

A major limiting factor in hydrocarbon (HC) biodegradation within phytoremediation systems is the toxicity of these hydrophobic pollutants, which restrict plant access to water and nutrients (Tesar et al., 2002; Peng et al., 2009; Wyszkowski and Ziólkowska, 2009). In contaminated soils, microbial populations and diversity decline drastically, resulting in low effectiveness of microorganisms in degrading hydrocarbons. Consequently, functional microbes that support the growth of phytoremediator plants become ineffective (Zhang et al., 2012). To overcome this limitation, selecting appropriate plant–microbe interactions is a viable strategy. Synergistic interactions have the potential to promote plant growth and stimulate hydrocarbon degradation processes (Weyens et al., 2009c; Tang et al., 2010; Fernandez et al., 2011; Muratova et al., 2009).

Several studies have demonstrated that synergistic interactions between plants and inoculated bacteria result in more efficient hydrocarbon rhizodegradation compared to microbial remediation alone or phytoremediation without bacterial inoculation (Afzal et al., 2012). Plant–bacteria interactions represent a promising strategy for restoring contaminated soils (Germaine et al., 2009; Weyens et al., 2009c, 2010b, 2010c; Glick, 2010; Afzal et al., 2012). Successful colonization of inoculated rhizobacteria in different plant compartments has been identified as a critical factor in improving phytoremediation efficiency (Nie et al., 2011; Afzal et al., 2012; Wang et al., 2012). Therefore, studies on the interaction, viability, and compatibility of plant growth–promoting rhizobacteria (PGPR) as biostimulants in phytoremediation systems are essential.

The combined application of plants and PGPR represents a relatively new concept in the remediation of petroleum hydrocarbon– contaminated soils (Zhuang et al., 2007; Afzal et al., 2012; Ahmad et al., 2012). Sorghum (Sorghum bicolor L.) is considered a promising phytoremediator plant due to its fibrous, strong, and extensively spreading root system. Root exudates released into the rhizosphere can increase microbial populations by 5–100 times, thereby enhancing hydrocarbon degradation (Estuningsih et al., 2012). An important factor to consider is the availability of nitrogen (N) and phosphorus (P) to support sorghum growth as a phytoremediation agent (Wahida, 2011). Previous studies have shown that sorghum used as a phytoremediator without the addition of biological agents achieved hydrocarbon biodegradation efficiencies ranging from 60–65% (Suryatmana et al., 2024b). Omara et al. (2019) reported that sorghum can survive in petroleum-contaminated soils; however, its phytoremediation capability was not evaluated in that study.

Nitrogen and phosphorus are essential nutrients in phytoremediation systems. The application of microorganisms capable of fixing atmospheric nitrogen ($N_2$) and increasing phosphorus availability (phosphate solubilizers) represents an effective approach to achieving adequate N and P conditions in petroleum hydrocarbon–contaminated phytoremediation systems. Among the potential PGPR candidates are Azotobacter vinelandii. and Pseudomonas sp.

Azotobacter spp. is an aerobic nitrogen-fixing bacterium capable of fixing substantial amounts of nitrogen (Suryatmana, 2006; Suytamana et al., 2024a), thereby potentially substituting nitrogen requirements during hydrocarbon biodegradation. Azotobacter vinelandii is known to secrete biosurfactant-like compounds (Suryatmana, 2006), which function as emulsifying, dispersing, and foaming agents (Thavasi, 2009). At population densities of $10^5$ CFU $mL^{-1}$, Azotobacter vinelandii. can also produce exopolysaccharides (EPS) and biosurfactants (Vermani et al., 1997), as well as phytohormones such as indole-3-acetic acid (IAA) and gibberellins, while fixing nitrogen (Salhia, 2010).

Pseudomonas sp. is a Gram-negative bacterium capable of solubilizing mineral-bound phosphate in soil into plant-available forms by releasing phosphorus from Fe, Al, Ca, and Mg complexes (Rao, 1992). Inoculation of Pseudomonas sp. into phytoremediation systems is expected to enhance hydrocarbon degradation, as Pseudomonas spp. are known to degrade both aliphatic and aromatic hydrocarbons (Syafrizal et al., 2010). Zhou et al. (2009) reported that soil phosphorus levels in hydrocarbon-contaminated soils are a critical parameter for effective phytoremediation. Moreover, Pseudomonas spp. are recognized as petrophilic microorganisms, and Suryatmana (2006) demonstrated that petrophilic consortia significantly increase the rate of petroleum hydrocarbon biodegradation.

The focus of this study was to evaluate the effects of Azotobacter vinelandii. and Pseudomonas sp. inoculation interactions and their contribution to sorghum plants in enhancing petroleum hydrocarbon biodegradation. The study examined several parameters, including patterns of petroleum waste biodegradation efficiency, viability of Azotobacter vinelandii. and Pseudomonas sp. during the biodegradation process, sorghum plant height growth, and hydrocarbon uptake by sorghum plants. This study aims to elucidate the potential of Azotobacter vinelandii. and Pseudomonas sp. in supporting sorghum growth and performance as a phytoremediation plant for petroleum-contaminated soils.

## 2.MATERIALS AND METHODS

### 2.1. Experimental Site

This study was conducted under greenhouse conditions at the Experimental Garden of the Faculty of Agriculture, Universitas Padjadjaran. The greenhouse is located at an altitude of 725 meters above sea level. Laboratory analyses were carried out at the Biology and Biotechnology Laboratory, Department of Soil Science, Faculty of Agriculture, Universitas Padjadjaran, Jatinangor, Sumedang Regency, West Java Province.

### 2.2. Materials

The materials used in this study included sorghum seeds of the Unpad 1.1 variety; Azotobacter vinelandii. and Pseudomonas sp. inoculants with a density of $10^7$ CFU $mL^{-1}$ obtained from the Soil Biology and Biotechnology Laboratory, Faculty of Agriculture, Universitas Padjadjaran; crude oil obtained from Pertamina Balongan, Indramayu; growth media for Azotobacter vinelandii. consisting of mineral liquid medium supplemented with 2% molasses as a carbon source; and Pikovskaya liquid medium for Pseudomonas sp. culture production.

Inorganic fertilizers were applied at 50% of the recommended dose, consisting of urea at 100 kg $ha^{-1}$ (equivalent to 0.5 g $pot^{-1}$), TSP at 50 kg $ha^{-1}$ (0.25 g $pot^{-1}$), KCl at 25 kg $ha^{-1}$ (0.125 g $pot^{-1}$), and cow manure compost applied at a rate of 5 tons $ha^{-1}$ (25 g $pot^{-1}$).

### 2.3. Experimental Design

The experiment was arranged using a factorial Randomized Block Design (RBD) with two factors. The first factor was Azotobacter vinelandii. inoculation at four levels, while the second factor was Pseudomonas sp. inoculation at four levels. Each treatment combination was replicated three times.

**Factor I: *Azotobacter vinelandii*. inoculation at different concentrations**

- $a_0$ = without *Azotobacter vinelandii.*
- $a_1$ = *Azotobacter vinelandii*. at 1% per TPH concentration
- $a_2$ = *Azotobacter vinelandii*. at 2% per TPH concentration
- $a_3$ = *Azotobacter vinelandii*. at 3% per TPH concentration

**Factor II: *Pseudomonas* sp. inoculation at different concentrations**

- $b_0$ = without *Pseudomonas* sp.
- $b_1$ = *Pseudomonas* sp. at 1% per TPH concentration
- $b_2$ = *Pseudomonas* sp. at 2% per TPH concentration
- $b_3$ = *Pseudomonas* sp. at 3%

per TPH concentration the experiment was conducted over a period of 14 weeks.

### 2.4. Propagation of Azotobacter vinelandii. and Pseudomonas sp. Cultures

The inoculum cultures of both bacteria were propagated using two different liquid media. A mineral liquid medium containing 2% molasses was used for Azotobacter vinelandii. biomass production, while Pikovskaya liquid medium was used for Pseudomonas sp. biomass production. The starter culture concentration used was 10% (v/v) of each medium volume. The cultures were inoculated into their respective media and incubated for 72 hours at 100 rpm.

**2.5. Preparation of Petroleum-Contaminated Soil**

The soil used in this experiment was first analyzed for its chemical and physical properties to determine its general characteristics. The soil sample was Inceptisols from Jatinangor, classified as Fluventic Eutrudepts, clayey, kaolinitic, isohyperthermic (Arifin et al., 2018). The soil was collected as a composite sample, air-dried, and sieved. Each pot contained 10 kg of soil, which was contaminated with crude oil at a concentration of 5% total petroleum hydrocarbons (TPH) on a weight-to-weight basis. The soil was thoroughly mixed to ensure uniform contamination. Compost was then added at a rate of 5 tons $ha^{-1}$ (25 g $pot^{-1}$) and mixed evenly. This medium was used for sorghum planting as part of the phytoremediation system. Basal fertilization was applied using inorganic fertilizers (urea, SP-36, and KCl) at 50% of the recommended dose. The prepared soil was inoculated with Azotobacter vinelandii. and Pseudomonas sp. cultures by applying the inoculants into the planting holes at concentrations of 0, 1%, 2%, and 3% liquid culture inoculum per waste load (soil TPH concentration), according to the treatments. One-month-old sorghum seedlings were then planted in each inoculated planting hole.

Plant maintenance included watering, replanting, weeding, and plant protection. Observations of response parameters were conducted at two-week intervals.

**2.6. Analysis of Total Petroleum Hydrocarbons and Viability of Azotobacter vinelandii and Pseudomonas sp. During Phytoremediation**

The reduction in hydrocarbon concentration was determined by analyzing residual TPH in the experimental soil using the n- hexane gravimetric method. The extraction process was carried out as follows: 10 g of soil sample was dissolved in n-hexane solvent in a TPH extraction bottle and shaken using a vortex mixer at maximum speed to obtain the TPH extract. The upper layer, containing petroleum dissolved in n-hexane, was transferred to a TPH sample container. The extraction process was repeated until all TPH was completely extracted from the soil particles, as indicated by the clear color of the n-hexane solvent. The extracted

TPH samples were evaporated at 45°C until the n-hexane was completely removed. The remaining TPH was weighed, and hydrocarbon degradation efficiency was calculated using the methods of Greenberg et al. (2007) and Pikoli (2000).

Hydrocarbon degradation efficiency was calculated using the following equation:

$$\text{ED (\%)} = \frac{[\text{TPH}]_{t0} - [\text{TPH}]_{tn}}{[\text{TPH}]_{t0}} \times 100$$

Where:

ED (%) = degradation efficiency

$[\text{TPH}]_{t0}$ = TPH concentration at the beginning of the experiment (before phytoremediation)

$[\text{TPH}]_{tn}$ = TPH concentration at time n after the phytoremediation process

The viability of Azotobacter vinelandii. and Pseudomonas sp. was analyzed using the serial dilution Total Plate Count (TPC) method at the beginning of the experiment and at two-week intervals.

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**2.7. Statistical Analysis**

Experimental data were analyzed using Statistical Product and Service Solutions (SPSS) version 15.0. Analysis of variance (ANOVA) was performed, and significant differences were determined at a 5% significance level ($p < 0.05$).

## 3. RESULT AND DISCUSSION

### 3.1 Characteristics of the Inceptisol

The characteristics of the Inceptisol soil used in this study are presented in detail in Table 1. The soil used as the planting medium was classified as Inceptisol and was collected from a depth of 0–20 cm below the soil surface.

**Table 1. Chemical and Physical Characteristics of the Experimental Soil**

| *No* | *Parameters* | | *Velue* | *criteria**) |
|---|---|---|---|---|
| 1. | pH H2O | | 5.98 | Slighly acid |
| 2. | pH KCl 1 N | | 5.24 | - |
| 3. | soil pH after contaminated | | 6.78 | Neutral |
| 4. | Organic-C | % | 2.29 | Moderate |
| 5. | Total-N | % | 0.16 | Low |
| 6. | C/N rasio | | 14.31 | Moderate |
| 7. | P2O5HCl 25% | mg 100 $g^{-1}$ | 56.28 | High |
| 8. | K2OHCl 25% | mg 100 $g^{-1}$ | 21.05 | Moderate |
| 9. | P2O5Bray I | ppm P | 4.75 | Low |
| | Cation exchange Capasity (CEC): | | | |
| | K | cmol $kg^{-1}$ | 0.26 | Low |
| | Na | cmol $kg^{-1}$ | 0.11 | Low |
| | Ca | cmol $kg^{-1}$ | 2.02 | Low |
| 10. | Mg | cmol $kg^{-1}$ | 0.66 | Moderate |
| 11. | KTK | cmol $kg^{-1}$ | 15.90 | Low |
| 12. | Base Saturated | % | 18.18 | Very low |
| 13. | Al-Saturated | % | 2.80 | Very low |
| 14. | $Al^{+3}$ dd (cmol/kg) | cmol/kg | 0.10 | |
| 15. | $H^{+}$ dd (cmol/kg) | cmol/kg | 0.41 | - |
| | Texture: | | | - |
| | Sand (%) | % | 7.00 | |
| | Silt (%) | % | 40.00 | |
| | Clay (%) | % | 53.00 | Silty Clay |

Source of criteria determination: *) Hardjowigeno (2007)

The initial soil analysis indicated the following characteristics: the soil had a slightly acidic pH (5.98), which increased to neutral (6.78) after petroleum contamination. The organic carbon content (2.29%) was classified as moderate, the cation exchange capacity (CEC) was low (15.90 cmol $kg^{-1}$), and the soil texture was silty clay. Organic carbon content was in the moderate category, available phosphate was low, and both CEC and base saturation were categorized as low.

The physical characteristics showed that the soil consisted of 7% sand, 40% silt, and 53% clay, classifying it as silty clay. Clay- textured soils have a high capacity to retain water and effectively bind molecules (Hardjowigeno, 2007; Arifin et al., 2018).

Based on these analytical results, the Inceptisol used as the planting medium exhibited low productivity. Therefore, appropriate soil management practices are required to improve soil productivity. The application of compost and NPK fertilizers was necessary to support the growth and development of sorghum plants as phytoremediation agents.

### 3.2 Hydrocarbon Degradation Efficiency

Statistical analysis of hydrocarbon degradation efficiency data showed that there was no interaction between the doses of Azotobacter vinelandii. and Pseudomonas sp. on hydrocarbon degradation efficiency. The independent effects of each treatment are presented in Table 2.

**Table 2. Effect of Azotobacter vinelandii. and Pseudomonas sp. to Hydrocarbon Biodegradation Efficiency**

| *Treatment* | *Hydrocarbon Biodegradation Efficiency (%)* |
|---|---|
| *Azotobacter* (A): | |
| a0= without *Azotobacter vinelandii*. | 68.165 a |
| a1= 1% *Azotobacter vinelandii.* | 76.656 b |
| a2 = 2% *Azotobacter vinelandii.* | 74.260 b |
| a3 = 3% *Azotobacter vinelandii.* | 75.817 b |
| *Pseudomonas*(B): | |
| b0 = without *Pseudomonas* sp. | 60.423 a |
| b1= 1% *Pseudomonas* sp. | 61.317 a |
| b2 = 2% *Pseudomonas* sp. | 72.613 b |
| b3 = 3% *Pseudomonas* sp. | 66.546 ab |

Note: Mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% significance level

There was no interaction between the inoculation of Azotobacter vinelandii. and Pseudomonas sp. on hydrocarbon degradation efficiency at week 14. However, an increase in degradation efficiency was observed as a result of the independent effects of each treatment.

The results of biodegradation analysis at week 14 after planting showed that hydrocarbon biodegradation efficiency increased significantly compared to the control treatment (without Azotobacter vinelandii. inoculation). This increase was attributed to the role of Azotobacter vinelandii. in the petroleum hydrocarbon degradation process. Azotobacter vinelandii. is known to possess adaptive capabilities to toxic stress caused by intermediate compounds formed during hydrocarbon degradation. Under stress conditions, Azotobacter tends to produce extracellular compounds that function as protective agents. These compounds also act as biosurfactants capable of emulsifying petroleum hydrocarbons into micelle-sized oil droplets. The formation of oil micelles enhances the absorption rate by hydrocarbon-degrading bacteria, thereby increasing the degradation rate and overall degradation efficiency.

As reported by Suryatmana et al. (2007), several genera of Azotobacter sp. produce extracellular substances that function as biosurfactants, which can support increased efficiency of petroleum hydrocarbon biodegradation.

The biodegradation efficiency values resulting from treatments with both biological agents indicate their potential performance in accelerating the biodegradation process of petroleum oil waste. However, no positive compatibility was observed when both agents were applied in combination. This finding contrasts with previous studies, such as Afzal et al. (2012), which reported that plant-associated bacteria, including rhizobacteria (RB), have been shown to contribute to the biodegradation of toxic organic compounds in contaminated soils and possess the potential to enhance phytoremediation (Siciliano et al., 2001; Germaine et al., 2009; McGuinness and Dowling, 2009; Weyens et al., 2009; Afzal et al., 2012).

In the present study, however, the roles of Azotobacter vinelandii. and Pseudomonas sp. did not exhibit synergistic interaction, either between the two bacterial species or in their interaction with plants in promoting hydrocarbon degradation, as no significant effects were observed. Several studies have reported that synergistic interactions between plants and bacterial communities are critical factors and are highly dependent on the survival and activity of exogenous bacteria carrying hydrocarbon-degrading genes required for the breakdown of organic pollutants (Glick, 2003; Muratova et al., 2008; Afzal et al., 2012). Therefore, as suggested by Johansson et al. (2009), it is essential to monitor population abundance and gene expression during the phytoremediation of contaminated soils in order to obtain stronger evidence of the effectiveness and functional activity of the inoculated microorganisms

### 3.3. Indigenous Petrobacter Population

The indigenous Petrobacter population plays a role in the hydrocarbon degradation process due to its ability to utilize carbon compounds. Statistical analysis showed that there was no interaction between the doses of Azotobacter vinelandii. and Pseudomonas sp. on the indigenous Petrobacter population. In addition, the independent effects of each treatment were not significant (Table 4), indicating that the levels of each treatment did not differ significantly from the control.

**Table 4. Effect of Azotobacter vinelandii. and Pseudomonas sp. on the Indigenous Petrobacter Population (t14)**

| *Treatment* | *Petrobacter indigenous population* ($10^3$CFU/g) |
|---|---|
| *Azotobacter* (A) | |
| $a_0$= without *Azotobacter vinelandii.* | 28.500 |
| $a_1$= 1% *Azotobacter vinelandii.* | 35.708 |
| $a_2$ = 2% *Azotobacter vinelandii.* | 36.000 |
| $a_3$ = 3% *Azotobacter vinelandii.* | 34.750 |
| *Pseudomonas*(B) | |
| $b_0$ = without *Pseudomonas* sp. | 30.750 |
| $b_1$= 1% *Pseudomonas* sp. | 34.708 |

| | |
|---|---|
| $b_2$ = 2% *Pseudomonas* sp.<br>$b_3$ = 3% *Pseudomonas* sp. | 42.500<br>27.000 |

Note: Mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% significance level.

The inoculation of Azotobacter vinelandii. and Pseudomonas sp. exhibited a tendency to increase the indigenous Petrobacter population; however, this effect was not statistically significant. The limited adaptive capacity of Azotobacter vinelandii. under toxic environmental conditions may have constrained its functional performance. As a result, the production of biostimulant compounds by Azotobacter vinelandii., including thiamine, riboflavin, pyridoxine, indole-3-acetic acid, gibberellins, and cytokinins (Suryatmana, 2006), may have been insufficient to effectively promote the growth of the indigenous Petrobacter population.

At week 14, both Pseudomonas sp. and Azotobacter vinelandii. were likely still undergoing adaptation to the utilization of recalcitrant carbon compounds. Under such conditions, microbial activity may have been primarily directed toward sustaining cellular metabolism rather than facilitating the proliferation of the indigenous Petrobacter population.

Indigenous Petrobacter populations possess several inherent advantages, including the ability to degrade contaminants following prolonged exposure and to adapt to locally contaminated environments, thereby enabling effective competition in polluted soils (Munawar, 2012). Nevertheless, in the present study, the indigenous Petrobacter population remained low, and the inoculation of Azotobacter vinelandii. and Pseudomonas sp. did not result in a significant enhancement of its population.

### 3.5 Growth of Sorghum Plants during the Phytoremediation Process

#### 3.5.1 Sorghum Plant Height at 2 Weeks After Planting (2 WAP)

Statistical analysis indicated that there was no interaction between the doses of Azotobacter vinelandii. and Pseudomonas sp. on sorghum plant height at 2 weeks after planting (2 WAP). However, the independent effects of each treatment were statistically significant with respect to plant height at 2 WAP (Table 5).

Successful seed germination is closely associated with the endosperm, which functions as a reservoir of stored nutrients. Seed reserves consist primarily of carbohydrates, lipids, proteins, and minerals that are mobilized and absorbed by the embryo before and/or during the germination process. Seed size also influences the nutritional content of the endosperm, as larger seeds generally contain greater nutrient reserves than smaller ones.

In this study, the application of Azotobacter vinelandii. and Pseudomonas sp. during seed germination resulted in a significant improvement in early plant growth at two weeks after planting under stress conditions induced by 5% total petroleum hydrocarbon (TPH) contamination, compared with the control treatment. The rapid hydrocarbon degradation observed during this period likely reduced soil toxicity, thereby creating more favorable conditions for seedling establishment. These results indicate that both bacterial inoculants functioned effectively under petroleum hydrocarbon stress

**Table 5. Effects of Azotobacter vinelandii. and Pseudomonas sp. on Sorghum Plant Height at 2 Weeks After Planting (2 WAP)**

| *Treatment* | *Plant Heigh at 2 WAP (cm)* |
|---|---|
| *Azotobacter* (A) | |
| $a_0$= without *Azotobacter vinelandii.* | 4.225 a |
| $a_1$= 1% *Azotobacter vinelandii.* | 8.100 b |
| $a_2$ = 2% *Azotobacter vinelandii.* | 9.025 b |
| $a_3$ = 3% *Azotobacter vinelandii.* | 7.592 b |
| *Pseudomonas*(B) | |
| $b_0$ = without *Pseudomonas* sp. | 6.008 a |
| $b_1$= 1% *Pseudomonas* sp. | 8.325 b |
| $b_2$ = 2% *Pseudomonas* sp. | 8.042 b |
| $b_3$ = 3% *Pseudomonas* sp. | 8.567 b |

Note: Mean values followed by the same letter are not significantly different according to Multiple Range Test at the 5% significance level.

According to Palleroni and Moore (2004), Pseudomonas sp. is capable of degrading and utilizing a wide range of organic and inorganic compounds, while also interacting with plants through rhizosphere associations, functioning as a plant growth– promoting rhizobacterium (PGPR). Plant growth was further enhanced by the inoculation of Azotobacter spp, which act as a biological agent producing phytohormones that can be utilized by plants (Suryatmana et al., 2024a). Similarly, Pseudomonas sp. has been widely reported to produce substantial amounts of phytohormones, particularly indole-3-acetic acid (IAA), which stimulate plant growth and contribute to hydrocarbon degradation (Suryatmana et al., 2022). In addition, Pseudomonas sp. functions as a phosphate-solubilizing bacterium, N-fixer and PGPR, thereby increasing phosphorus availability for plant uptake (Suryatmana et al., 2022).

### 3.5.2 Sorghum Plant Height Growth during the Degradation Process (14 Weeks After Planting)

The growing environment of sorghum plants was subjected to extreme conditions due to the presence of petroleum hydrocarbons containing toxic aromatic and cycloparaffinic compounds (Suryatmana, 2006), which may inhibit plant height growth. Statistical analysis showed that there was no interaction between the doses of Azotobacter vinelandii. and Pseudomonas sp. on sorghum plant height at 14 weeks after planting (14 WAP) (Table 6).

**Table 6. Effects of Azotobacter vinelandii. and Pseudomonas sp. on Sorghum Plant Height at 14 Weeks After Planting (14 WAP)**

| *Treatment* | *Plant Height at 14 MST (cm)* |
|---|---|
| *Azotobacter* (A) | |
| $a_0$= without *Azotobacter vinelandii.* | 10.192 |
| $a_1$= 1% *Azotobacter vinelandii.* | 9.250 |
| $a_2$ = 2% *Azotobacter vinelandii.* | |

| | |
|---|---|
| $a_3$ = 3% *Azotobacter vinelandii.* | 8.631<br>8.732 |
| *Pseudomonas*(B) | |
| $b_0$ = without *Pseudomonas* sp. | 10.708 b |
| $b_1$= 1% *Pseudomonas* sp. | 8.883 a |
| $b_2$ = 2% *Pseudomonas* sp. | 11.500 b |
| $b_3$ = 3% *Pseudomonas* sp. | 9.533 ab |

Note: Mean values followed by the same letter are not significantly different according to Multiple Range Test at the 5% significance level.

No interaction was observed between Azotobacter vinelandii. and Pseudomonas sp. on sorghum plant height at 14 weeks after planting. This outcome may be attributed to the limited adaptive capacity of both inoculated bacteria under the prevailing environmental conditions at week 14, when more recalcitrant hydrocarbon fractions dominated the contaminated medium. Under such conditions, both sorghum growth and the activity of the inoculated bacteria were likely subjected to physiological stress.

The reduced performance of Azotobacter vinelandii. and Pseudomonas sp. under these conditions may have constrained the supply of bioavailable nitrogen, solubilized phosphate, biosurfactants required for hydrocarbon solubilization, and phytohormones essential for promoting sorghum height growth. Consequently, the beneficial effects of bacterial inoculation on plant growth were diminished.

The suboptimal performance of Azotobacter vinelandii. observed in this study is consistent with the findings of Rao (1994), who reported that the effects of Azotobacter sp. application can vary widely, ranging from no significant response to a marked enhancement of plant growth. Accordingly, sorghum plant height in this phytoremediation experiment did not show a significant increase compared with the control treatment up to 14 weeks after planting, corresponding to the harvest stage of the crop.

## 4.CONCLUSION

No interaction was observed between Azotobacter vinelandii. and Pseudomonas sp. across all variables evaluated, indicating that these two bacterial species are not compatible when applied as a consortium but instead exert independent effects. The independent inoculation of Azotobacter vinelandii. significantly increased total petroleum hydrocarbon (TPH) removal efficiency compared with the control at application rates of 1%, 2%, and 3% (based on TPH load) at 14 weeks after planting, resulting in biodegradation efficiencies of 68.160%, 76.656%, 74.260%, and 75.817%, respectively. In contrast, the independent effect of Pseudomonas sp. inoculation at 14 weeks after planting resulted in a significant increase in hydrocarbon biodegradation efficiency only at the 2% dose, achieving an efficiency of 72.613%. Both inoculants demonstrated potential as biostimulants capable of enhancing the biodegradation efficiency of petroleum hydrocarbon waste. However, neither inoculant contributed to an increase in the indigenous Petrobacter population nor promoted sorghum plant height growth; instead, a tendency toward growth inhibition was observed. Furthermore, the compatibility of both inoculants with sorghum plants did not show a positive interaction. Nevertheless, when applied independently, Azotobacter vinelandii. and Pseudomonas sp. functioned as effective biostimulants in promoting petroleum hydrocarbon degradation due to their roles as hydrocarbon-degrading bacteria, nitrogen fixers, and plant growth–promoting rhizobacteria (PGPR).

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