

# Enhancing the Phytoremediation Performance of Sorghum bicolor in Petroleum-Contaminated Soil via Dual-Function Azotobacter sp. Inoculation

Pujawati Suryatmana

Department of Soil Science and Land Resources, Agriculture Faculty, Universitas Padjadjaran

Wulan Feitriani

Agortechnology Programe Study, Agriculture Faculty of Universitas Padjadjaran

Mieke Rochimi Setiawati

Department of Soil Science and Land Resources, Agriculture Faculty, Universitas Padjadjaran

Arga Ritztama

Independent Researcher

他

<https://doi.org/10.5109/7395494>

---

出版情報 : Proceedings of International Exchange and Innovation Conference on Engineering & Sciences (IEICES). 11, pp.32-37, 2025-10-30. International Exchange and Innovation Conference on Engineering & Sciences

バージョン :

権利関係 : Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International



## Enhancing the Phytoremediation Performance of *Sorghum bicolor* in Petroleum-Contaminated Soil via Dual-Function *Azotobacter* sp. Inoculation

Pujawati Suryatmana<sup>1</sup>, Wulan Feitriani<sup>2</sup>, Mieke Rochimi Setiawati<sup>3</sup>, Arga Riztama<sup>4</sup>  
and Wahyudi Widyatmoko Parnadi<sup>5</sup>

<sup>1,3)</sup> Department of Soil Science and Land Resources, Agriculture Faculty,  
Universitas Padjadjaran, Indonesia

<sup>2)</sup> Agortechnology Programe Study, Agriculture Faculty of Universitas Padjadjaran, Indonesia

<sup>4)</sup> Independent Researcher, Jalan Cihampelas no.10 Bandung, West Java, Indonesia

<sup>5)</sup> Geophysical Engineering Department, Bandung Institute of Technology,  
Jl. Ganesha 10, Bandung 40132, Indonesia

Correspondence author: [pujawati@unpad.ac.id](mailto:pujawati@unpad.ac.id)

**Abstract:** Phytoremediation represents a promising and environmentally sustainable approach for the remediation of petroleum-contaminated soils. The effectiveness of plant-based remediation strategies is largely determined by the complex interactions among plants, rhizosphere microbial communities, and organic contaminants. This study assessed the *Azotobacter* sp. inoculation effect on the phytoremediation performance of *Sorghum bicolor* grown in petroleum-contaminated soil. A factorial experiment was conducted using a completely randomized design, incorporating treatments without inoculation and with inoculation of 1%, 2%, and 3% (v/wt.) *Azotobacter* sp. The findings revealed that 1% *Azotobacter* sp. inoculation significantly promoted sorghum plant height, while 2% *Azotobacter* sp. inoculation markedly enhanced petroleum hydrocarbon degradation efficiency, achieving 78.41%. Furthermore, 1% *Azotobacter* sp. inoculation was found to be compatible with the indigenous *Petrobacter* community, maintaining its population density. *Azotobacter* sp. supported petroleum biodegradation and promoting plant growth. *Azotobacter* sp. acts as both a biosurfactant-producing bacterium and a PGPR, further contributing to enhance phytoremediation efficiency.

**Keywords:** *Azotobacter* sp; Biodegrading Efficiency; Plant growth promoting rhizobacteria (PGPR)

### 1. INTRODUCTION

Petroleum hydrocarbon contamination in soils, sediments, and marine environments constitutes a significant global environmental threat, primarily stemming from the processes associated with crude oil and natural gas exploration, drilling, development, and production [6, 14]. Upon release into the environment, whether through accidental spills or routine operations, petroleum hydrocarbons tend to accumulate in soils and sediments, bringing with them a variety of pollutants such as benzene, toluene, ethylbenzene, xylenes (BTEX), as well as aliphatic and polycyclic aromatic hydrocarbons (PAHs) [14]. As described by Ite [14], petroleum, or crude oil, is a naturally occurring, chemically complex, and heterogeneous mixture predominantly composed of hydrocarbons.

Phytoremediation is a remediation strategy that utilizes plants to clean up contaminated environments, predominantly soils but also aquatic systems. Several mechanisms contribute to pollutant removal, including phytovolatilization (the emission of volatile compounds via plant tissues), Phyto-transformation (the chemical alteration of contaminants), Phyto-stabilization (the immobilization of pollutants within the soil matrix), and phytoextraction (the absorption and accumulation of trace elements within plant tissues), and Rhizoremediation. While all these processes depend on the interaction between plants and their associated microbial communities. Rhizoremediation focuses on the degradation of organic pollutants within the rhizosphere—the soil region influenced by root activity—where plant root exudates stimulate microbial

processes [19]. Rhizosphere microbes, particularly bacteria, are considered the primary agents responsible for degrading organic contaminants during phytoremediation/ rhizoremediation [2, 8].

The application of plant growth-promoting rhizobacteria (PGPR) is well-established for enhancing plant development [17], with particular emphasis on strains that stimulate root growth, which are especially beneficial for rhizoremediation efforts. In addition to promoting plant growth through hormone production and nutrient mobilization, PGPR also play a crucial role in mitigating plant stress via various mechanisms [7, 21], which helps plants maintain growth and resilience under highly contaminated conditions by lessening stress-induced effects [5].

Promoting root system development is a critical factor for enhancing the effectiveness of rhizoremediation. Therefore, any intervention that stimulates root growth is likely to improve rhizoremediation outcomes. PGPR exert their effects through a variety of mechanisms, and remains relatively underexplored [6], including the production of phytohormones such as indole-3-acetic acid (IAA) [22, 24] and gibberellic acid [10], while some contribute to plant health by outcompeting or suppressing soil-borne pathogens [3].

One of well-known genus of plant growth-promoting rhizobacteria (PGPR) is *Azotobacter*. This genus is recognized for its potential as a biosurfactant-producing bacterium and has been utilized as a co-inoculant to enhance the rate of hydrocarbon biodegradation [25]. Co-inoculation with *Azotobacter chroococcum* has been

shown to improve the biodegradation efficiency of crude oil hydrocarbons, increasing degradation rates within *Petrobacter* cultures [26]. *Azotobacter* species can function as bio-stimulants, providing benefits to *Petrobacter* by producing fatty acid compounds that act as biosurfactants, thereby facilitating the breakdown of petroleum hydrocarbon chains [25]. According to Jnawali [15], *Azotobacter* also excretes indole-3-acetic acid (IAA), gibberellins, and cytokinin, which serve as bio-stimulants for promote root growth in sorghum during the biodegradation process.

A particularly noteworthy aspect of *Azotobacter* is that research has shown *Azotobacter* spp. secrete extracellular compounds that function as biosurfactants. Biosurfactants play a critical role in enhancing the emulsification degree of petroleum pollutants, transforming “large droplet oil” structures into “micelle-sized oil” particles [25, 28]. This emulsified petroleum condition is crucial, as it enables *Petrobacter* to more readily absorb and degrade petroleum hydrocarbons, thereby accelerating the degradation process. As a result, the efficiency of petroleum hydrocarbon biodegradation is significantly increased.

Choosing a suitable plant species is critical to maximizing the effectiveness of rhizoremediation. Important criteria for plant selection include root system architecture, the plant’s tolerance to environmental contaminants, and the nature of its root exudates [6]. Plant root exudation plays a central role in promoting microbial degradation of petroleum hydrocarbons in the rhizosphere [14].

This study aimed to evaluate the effectiveness of *Azotobacter* sp. inoculation as a bio-stimulant to enhance the phytoremediation efficiency of hydrocarbon-contaminated soils using *Sorghum bicolor* as the phytoremediator, while simultaneously promoting the growth and development of the sorghum plant.

## 2. MATERIAL AND METHOD

This research was carried out at the Soil Biology and Microbiology Laboratory and the Experimental Farm of the Faculty of Agriculture, Universitas Padjadjaran, located in Jatinangor, Sumedang Regency, West Java, at an elevation of 745 meters above sea level. The materials utilized in this study included: Jatinangor Inceptisol soil, collected as a composite sample from the topsoil layer (0–20 cm depth), which served as the planting medium; *Sorghum bicolor* seeds of the Unpad 1.1 variety, obtained from the Plant Breeding Laboratory, Faculty of Agriculture, Universitas Padjadjaran; and *Azotobacter* sp. inoculants with a density of  $10^7$  CFU/g soil. Petroleum sludge waste containing 30% total petroleum hydrocarbons (TPH), provided by Balongan Pertamina, Indramayu, was used as the pollutant source. Additionally, Glucose Basal Medium was employed for the cultivation of *Azotobacter* sp. Compost was applied as the base fertilizer.

### 2.1. Experimental Design

The experiment was arranged using a Randomized Block Design (RBD) with three levels, specifically:

*Azotobacter* sp. treatment (A), with three levels as follows:

- a0 = without *Azotobacter* sp.
- a1 = 1 % *Azotobacter* sp./ waste load

- a2 = 2 % *Azotobacter* sp./ waste load
- a3 = 3% *Azotobacter* sp./ waste load

The total number of treatment combinations was  $4 \times 3 = 12$ , with each combination replicated three times.

### 2.2. Observed Parameters

The parameters observed included the initial and final soil pH, total petroleum hydrocarbon (TPH) content in the soil, the population density of *Azotobacter* sp. (CFU/g), sorghum plant growth indicators such as plant height (cm).

### 2.3. Cultivation of *Azotobacter* sp.

A 5% (v/v) stock culture of *Azotobacter* sp. was inoculated into basal medium within a 2-liter batch reactor. The culture was incubated for seven days until the bacterial population reached a density of  $10^7$  CFU/mL

### 2.4. Preparation of the Phytoremediation System.

The soil used in this study consisted of Inceptisol collected from the topsoil layer (0–20 cm) in Jatinangor. For each treatment, 10 kg of soil was placed into 12 kg capacity pots. Petroleum sludge obtained from Balongan Pertamina, Indramayu, was incorporated at a concentration of 5% relative to the soil weight and thoroughly homogenized. Subsequently, the soil was inoculated with a culture of *Azotobacter* sp. at a density of  $10^7$  CFU/mL, according to the respective treatment specifications. Fourteen-day-old *Sorghum* seedlings were then transplanted into the prepared contaminated soil. Compost was applied at a rate of 25 g per pot (equivalent to 5000 kg/ha), supplemented with 1 g urea per pot (100 kg/ha), 0.5 g TSP per pot (50 kg/ha), and 0.25 g KCl per pot (25 kg/ha). Routine maintenance included daily morning watering to prevent dehydration, replanting, weeding, and pest and disease management.

### 2.5. Analysis of Soil Total Petroleum Hydrocarbon (TPH) and Sorghum Growth Measurements

Soil samples (5 g) were collected from each pot at the second and sixteenth weeks for TPH analysis using the gravimetric method. Biodegradation efficiency was subsequently determined based on TPH concentration values. Plant height measurements were recorded biweekly from transplanting to harvest. At the sixteenth week, the shoot-to-root ratio of sorghum was assessed using Garner’s (1998) method. The population densities of *Petrobacter* (CFU/g) and *Azotobacter* spp. were quantified via the serial dilution plate count method at the second and sixteenth weeks post-treatment.

### 2.6. Calculation of Petroleum Sludge Biodegradation Efficiency (%)

Biodegradation efficiency was calculated based on TPH concentrations, determined through n-hexane extraction analysis conducted at the second and sixteenth weeks following treatment.

The Total Petroleum Hydrocarbon (TPH) value was calculated using the following formula:

$$\text{TPH} = \frac{(B-A)}{\text{Sample weigh}} \times 100\%$$

$$\text{Degradation Efficiency (DE)} = \frac{(X-Y)}{X} \times 100\%$$

**Legend:**

- **X** = Initial TPH (TPH at t-0)
- **Y** = TPH at t-n
- **A** = Initial weight of the vial
- **B** = Weight of the vial after oven drying and oil extraction post-evaporation

**2.7. Statistical Test**

Differences between treatments were analyzed using an F-test at a 5% significance level, while differences in mean values were evaluated using Duncan's Multiple Range Test at the 5% level.

**3. RESULTS AND DISCUSSION**

The soil employed in this research was an Inceptisol, collected from the experimental farm in Jatinangor as a composite sample from the 0–20 cm surface layer. The physicochemical characteristics of Inceptisol represented in Table 1.

Table 1. Physicochemical Characteristics of Inceptisol in the Jatinangor Area

No	Parameters	Unit	Value	Criteria
1.	pH H <sub>2</sub> O		5,85	Slightly acidic
2.	pH KCl 1 N		5,06	-
3.	C-Organic	%	1,44	Low
4.	N-total	%	0,32	Low
5.	C/N rasio		4	Very low
6.	P <sub>2</sub> O <sub>5</sub> Olsen	mg kg <sup>-1</sup>	5,12	Very low
7.	P <sub>2</sub> O <sub>5</sub> HCl	mg 100 g <sup>-1</sup>	5,70	Very low
	25%			

Preliminary analysis showed that the Inceptisol had a slightly acidic pH (5.85), a clay-heavy texture (47% clay content), and in very low levels of organic carbon, nitrogen and available phosphorus (P<sub>2</sub>O<sub>5</sub>). The concentrations of exchangeable cations such as Ca, K, Na, and H were generally low. This soil is categorized as a marginal soil. It is needed to improve by organic material amendment to enhance microorganism community biodiversity. According to Hindersah [12] reported that the population and diversity of soil bacteria higher in the soil which has higher organic C content, as like in top soil. Bulfa [11] also reported that the different carbon-to-nitrogen (C/N) ratios influenced the chemical characteristics and recovery efficiency of compost used.

**3.1. Components of Biosurfactant Compounds Produced by *Azotobacter* sp. with the Highest Emulsification Capacity**

Table 2 presents the analysis results of extracellular compounds produced by *Azotobacter* sp. cultured in a biosurfactant production medium containing glucose as the primary carbon source. The *Azotobacter* sp. culture used as an inoculum in the phytoremediation system was found to produce extracellular compounds functioning as petroleum biosurfactants. Chromatographic analysis identified the fatty acid components of the biosurfactant Az, synthesized from the optimal carbon source, glucose. The biosurfactant components produced by *Azotobacter* sp., which exhibited the highest emulsification index (E<sub>24</sub>), are composed of various fatty acids. Testing

biosurfactant quality across different carbon sources revealed that glucose-based biosurfactants produced the best emulsification performance, serving as the baseline for further biosurfactant production and application studies using *Azotobacter* sp. culture produced biosurfactant.

Table 2. Analysis Results of the Biosurfactant Compound Composition Produced by *Azotobacter* sp.

No	Compounds	Chemical Formulas	% Total abundant
1	Dodecanoic acid	C <sub>12</sub> H <sub>24</sub> O <sub>2</sub>	7,28
2	1-heptanol,2-propyl-	C <sub>10</sub> H <sub>22</sub> O	1,11
3	Octadecanoic acid (CAS)	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	6,49
4	1-undecene,8-methyl-	C <sub>12</sub> H <sub>24</sub>	1,17
5	Methyl arachate	C <sub>21</sub> H <sub>42</sub> O <sub>2</sub>	1,92
6	n-hexadecenoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	45,39
7	Oleoyl	C <sub>18</sub> H <sub>36</sub> O	1,62
8	9-hexadecenoic acid (CAS)	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	7,93
9	9-hexadecenoic acid (CAS)	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	18,47
10	Red oil	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	6,54
11	1-undecene, 8-methyl-	C <sub>12</sub> H <sub>24</sub>	1,14
12	Hexane,1,3-epoxy-	C <sub>6</sub> H <sub>12</sub> O	0,93

The composition of fatty acids in *Azotobacter* biosurfactants is a key determinant of their emulsification quality and index. The biosurfactant compounds produced through fermentation with glucose as the carbon source consisted of 12 different fatty acids, demonstrating a significantly higher hydrocarbon emulsification capacity compared to those derived from mannitol-based fermentation (data not shown).

The biosurfactant produced by *Azotobacter* sp. was predominantly composed of hexadecenoic acid, dodecanoic acid, octadecanoic acid, and Red Oil. These compounds are amphipathic in nature, contributing to their strong biosurfactant properties.

The production process of biosurfactant at a 5-liter scale was conducted using a medium containing glucose as the sole carbon source. The selection of glucose was based on prior testing, which identified it as the optimal carbon source for producing biosurfactants with the highest quality (see Fig 1).



Fig 1. Biosurfactant Production by *Azotobacter* sp. culture used in Phytoremediation treatment.

### 3.2. Total Population Density of Indigenous *Petrobacter* and *Azotobacter* spp.

Statistical analysis revealed that the addition of *Azotobacter* sp. during the phytoremediation of petroleum sludge contributed to maintain in the population density of indigenous *Petrobacter* (see Fig 2).

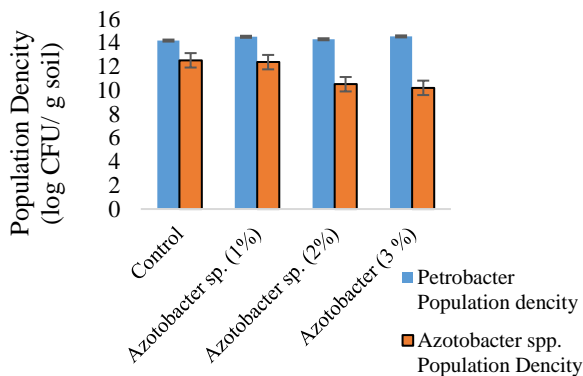


Fig 2. *Petrobacter* population density compared by *Azotobacter* spp. Density during biodegradation hydrocarbon process as long as 16 weeks incubation.

The results of this study demonstrated that the application of *Azotobacter* sp. did not significantly increase the total population density of *Petrobacter*. However, the treatment with 1% *Azotobacter* sp. maintained *Petrobacter* growth without inhibition, suggesting a compatible interaction between the two microbial groups. In contrast, augmentation with 2% and 3% *Azotobacter* sp. appeared to cause a slight decline in the population density of indigenous *Azotobacter* spp., although no reduction in *Petrobacter* population density was observed. This phenomenon may be attributed to competition for available nutrients between the augmented *Azotobacter* sp. (at concentrations exceeding 1%) and the native *Azotobacter* community present in the soil. Tao [27] similarly reported that competition for resources, such as carbon and energy sources, can occur between introduced microbial strains and indigenous microbial communities.

### 3.3. Effect of *Azotobacter* sp. Inoculation on Hydrocarbon Degradation Efficiency in Petroleum-Contaminated Soil

*Azotobacter* sp. inoculation in the phytoremediation process of petroleum sludge significantly increases degradation efficiency compared to the control treatment (see Fig 3).

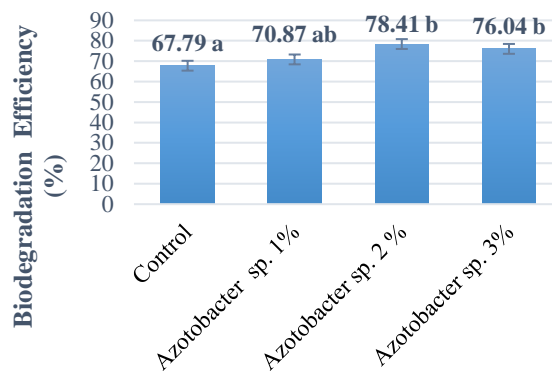


Fig 3. Efficiency of hydrocarbone biodegradatio during Phytoremediation process by *Sorghum bicolor* using *Azotobacter* sp. augment as long as 16 weeks incubation.

The inoculation of *Azotobacter* sp. at concentrations of 2% and 3% significantly enhanced the efficiency of petroleum sludge hydrocarbon degradation (78.42% and 76.04% respectively) compared to the uninoculated (control treatment). This improvement is primarily attributed to the ability of *Azotobacter* sp. to promote the emulsification of petroleum sludge, facilitating its use as an immediate carbon source that supports *Petrobacter* catabolic activity, leading to ATP production for microbial growth. *Petrobacter* spp. aerobically degrade hydrocarbons present in petroleum sludge, thereby contributing to the observed increase in degradation efficiency.

The hydrocarbon-degrading capability of *Petrobacter* is further supported by root exudates from *Sorghum bicolor*, the phytoremediator plant used in this study. In addition to its role in emulsification, *Azotobacter* sp. functions as a plant growth-promoting rhizobacterium (PGPR), stimulating root growth and enhancing the release of root exudates. These exudates create a favorable rhizosphere environment that accelerates the degradation of toxic organic compounds and provide additional substrates for microbial communities [18]. Pivetz [20] similarly reported that root exudates—including sugars, amino acids, organic acids, fatty acids, sterols, growth factors, nucleotides, flavonoids, enzymes, and other metabolites—stimulate microbial proliferation and activity within the rhizosphere. Consequently, this enhances the activity of petrophylic bacteria such as *Petrobacter*, ultimately leading to greater petroleum hydrocarbon degradation.

The findings of this study confirmed that *Azotobacter* sp. significantly enhances phytoremediation performance in petroleum-contaminated soils compared to the control. This enhancement can be attributed to two main mechanisms.

First, *Azotobacter* sp. produces biosurfactant compounds that increase hydrocarbon solubility in the soil matrix [26]. These biosurfactants facilitate the dispersion of hydrocarbon pollutants into micelle-sized droplets, reducing interfacial tension between microorganisms and hydrocarbons [4]. This process promotes the adsorption of micelle-sized oil droplets by indigenous petrophylic microbes, improving the desorption and bioavailability of polycyclic aromatic hydrocarbons (PAHs). As a result, indigenous

petrophylic microorganisms can more readily adsorb micelle-sized oil droplets, thereby improving desorption efficiency and enhancing the bioavailability of polycyclic aromatic hydrocarbons (PAHs) [9, 23]. Additionally, *Azotobacter vinelandii* has been reported to biodegrade complex hydrocarbons, such as the transformation of decahydro-2-methyl groups into simpler dimethyl compounds [26].

Second, *Azotobacter* sp. acts as a biofertilizer by providing essential nutrients that further support bioremediation [13, 26]. As a nitrogen-fixing bacterium, *Azotobacter* spp. can contribute approximately 20 kg of fixed nitrogen per hectare annually [16], thus promoting the growth of *Sorghum bicolor* and enhancing its effectiveness as a phytoremediator.

### 3. 4. Increase in Sorghum Plant Height

Statistical analysis results indicate that the inoculation of *Azotobacter* sp. in the phytoremediation process of petroleum sludge significantly increased the height of sorghum plants (see Fig 4).

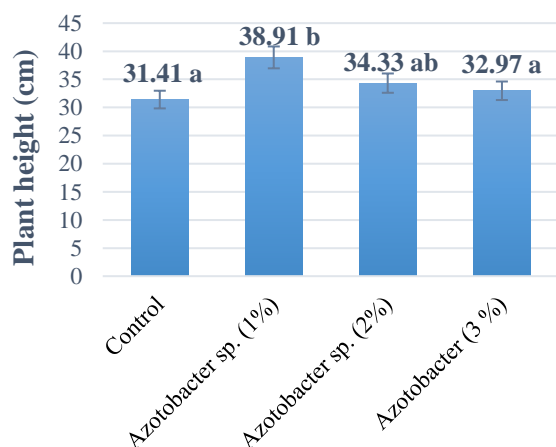


Fig 4. Efficiency of hydrocarbon biodegradation during Phytoremediation process by *Sorghum bicolor* using *Azotobacter* sp. augmented as long as 16 weeks incubation

The application of 1% *Azotobacter* sp. significantly increased the height of sorghum plants (38.92 cm) compared to the control treatment, although it was not markedly different from the other treatments. Based on descriptions, the sorghum variety Unpad 1.1 exhibited an average height of approximately 135 cm, which was substantially higher than the mean final height of plants subjected to various treatments. This result is likely influenced by the presence of petroleum hydrocarbon compounds absorbed by the plants, which negatively affected plant growth. Petroleum waste acts as a limiting factor for sorghum growth by impeding the plants' ability to absorb water and minerals, as the bound contaminants reduce mineral solubility and nutrient availability in the soil. Consequently, the plants became stunted and developed a suboptimal canopy structure, limiting their capacity to capture sunlight. However, analysis indicated that the application of *Azotobacter* sp. 1% significantly enhanced plant height, suggesting that *Azotobacter* can function as a plant growth-promoting rhizobacterium (PGPR) that supports better plant growth even under

hydrocarbon pollutant stress conditions. *Azotobacter* sp., as a biosurfactant-producing bacterium, acts as an emulsifier for petroleum hydrocarbons and also assists in supplying nitrogen, which is beneficial for sorghum growth. These findings are consistent with the study by Suryatmana [25], which demonstrated that *Azotobacter vinelandii* can serve as a substitute for nitrogen fertilizers while producing biosurfactants. Al-Jawhari [1] reported that the aerial height of wheat plants reached 73 cm following soil treatment with *Azotobacter* spp.

## 4. CONCLUSION

The hydrocarbon degradation process showed a tendency for the soil pH to move toward neutrality. The inoculation of *Azotobacter* sp. at a 1% (v/wt) dosage in phytoremediation using sorghum significantly increased the sorghum plant height, and application of 2% *Azotobacter* sp. significantly increased the efficiency of petroleum hydrocarbon biodegradation. The augmented *Azotobacter* sp. played a dual functional role: first, as a biosurfactant-producing agent that enhanced the emulsification of petroleum hydrocarbons, thereby improving their biodegradation efficiency; and second, as a plant growth-promoting rhizobacterium (PGPR) that stimulated plant growth, increased root exudate production, and subsequently activated *Petrobacter* activity for enhanced petroleum hydrocarbon degradation.

## 5. REFERENCES

- [1] I.F.H. Al-Jawhari, A.I. Flayyih, K.J. Al-Mansor, and D.Q. Mohammed. Bioremediation of crude oil and its effect of residue in growth of wheat plants. *GSC Advanced Research and Reviews* 13(2022), 188–199
- [2] H. T. Bell, J. Simon, E. P. Frédéric, and E. Yergeau. Increasing Phytoremediation Efficiency and Reliability Using Novel Omics Approaches.” *Trends in Biotechnology*. 32 (2014) 271–280. <https://doi.org/10.1016/j.tibtech.2014.02.008>.
- [3] A. Beneduzi, A. Ambrosini, and L.M.P. Passaglia. Plant Growth-Promoting Rhizobacteria (PGPR): Their Potential as Antagonists and Biocontrol Agents.” *Genetics and Molecular Biology*, (2012). 35 (4 suppl 1) 1044–51. <https://doi.org/10.1590/S1415-47572012000600020>.
- [4] N.K. Bordoloi, and B.K. Konwar. 2009. Bacterial Biosurfactant in Enhancing Solubility and Metabolism of Petroleum Hydrocarbons. *Journal of Hazardous Materials*. 170 (2009) 495–505. <https://doi.org/10.1016/j.jhazmat.2009.04.136>.
- [5] G.I. Burd, D.G. Dixon, and B.R. Glick. Plant Growth-Promoting Bacteria That Decrease Heavy Metal Toxicity in Plants. (2000). <https://doi.org/10.1139/w99-143>.
- [6] S. Correa-García, P. Pande, A. Séguin, Marc St-Arnaud, and E. Yergeau. 2018. Rhizoremediation of Petroleum Hydrocarbons: A Model System for Plant Microbiome Manipulation. *Microbial Biotechnology*. 11 (2018) 819–832. <https://doi.org/10.1111/1751-7915.13303>.
- [7] A. De Zelicourt, M. Al-Yousif, and H. Hirt. Rhizosphere Microbes as Essential Partners for Plant Stress Tolerance. *Molecular Plant*. 6 (2013) 242–245.

- <https://doi.org/10.1093/mp/sst028>.
- [8] A. El Amrani, A.S. Dumas, L.Y. Wick, E. Yergeau, and R. Berthomé. Omics' Insights into PAH Degradation toward Improved Green Remediation Biotechnologies. *Environmental Science & Technology* 49 (2015) 11281–11291. <https://doi.org/10.1021/acs.est.5b01740>.
- [9] J. Gómez, M.T. Alcántara, M. Pazos, and M.A. Sanromán. Remediation of Polluted Soil by a Two-Stage Treatment System: Desorption of Phenanthrene in Soil and Electrochemical Treatment to Recover the Extraction Agent. *Journal of Hazardous Materials* 173 (2010) 794–798. <https://doi.org/10.1016/j.jhazmat.2009.08.103>.
- [10] M. Gutiérrez, J. Francisco, B.R. Solano, A. Probanza, J. Mehouchi, F. R. Tadeo, and . Talon. The Plant-growth-promoting Rhizobacteria *Bacillus Pumilus* and *Bacillus Licheniformis* Produce High Amounts of Physiologically Active Gibberellins." *Physiologia Plantarum* 111 (2001) 206–11. <https://doi.org/10.1034/j.1399-3054.2001.1110211.x>.
- [11] IEICES website: <http://www.tj.kyushu-u.ac.jp/en/igses/ieices/> 10, pp. 11 - 18, 2024-10-17 (accessed 25.05.11).
- [12] IEICES website: <http://www.tj.kyushu-u.ac.jp/en/igses/ieices/> 10, pp. 204 - 210, 2024-10-17 (accessed 25.05.11).
- [13] H.T. Hoa, C. L. Wang, and C. H. Wang. The Effects of Different Substrates on the Growth, Yield, and Nutritional Composition of Two Oyster Mushrooms (*Pleurotus Ostreatus* and *Pleurotus Cystidiosus*). *Mycobiology* 43 (2015) 423–434. <https://doi.org/10.5941/MYCO.2015.43.4.423>.
- [14] E.A. Ite, and U.J Ibok. Role of Plants and Microbes in Bioremediation of Petroleum Hydrocarbons Contaminated Soils. *International Journal of Environmental Bioremediation & Biodegradation* 7 (2019) 1–19. <https://doi.org/10.12691/ijebb-7-1-1>.
- [15] A.D. Jnawali, R.B. Ojha, and S. Marahatta . Role of *Azotobacter* in soil fertility and sustainability—a review . *Adv Plants Agric Res* 2(2015) 250–253.
- [16] R. Kizilkaya. Nitrogen fixation capacity of *Azotobacter* spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. *J Environ Biol* 30(2009) 73–82.
- [17] J.W. Kloepper, and M.N. Schroth. 1978. Plant Growth Promoting Rhizobacteria on Radishes. In *Proceedings of the IVth International Conference on Plant Pathogenic Bacteria*, 2(1978) 879–882.
- [18] M. Palmroth. Phytoremediation of Subarctic Soil Contaminated with Diesel Fuel. *Bioresource Technology* 84 (2002) 221–28. [https://doi.org/10.1016/S0960-8524\(02\)00055-X](https://doi.org/10.1016/S0960-8524(02)00055-X).
- [19] E. Pilon-Smits. Phytoremediation. *Annual Review of Plant Biology* 56(2005)15–39.
- [20] B.E. Pivetz. Phytoremediation of Contaminated Soil and Ground Water at Hazardous Waste Sites United States Environmental Protection Agency. 2001.
- [21] M. Rajkumar, S. Sandhya, M.N.V. Prasad, and H. Freitas. Perspectives of Plant-Associated Microbes in Heavy Metal Phytoremediation. *Biotechnology Advances* 30 (2012) 1562–74. <https://doi.org/10.1016/j.biotechadv.2012.04.011>.
- [22] V. Raut, I. B. Shaikh, Naphade, K. Prashar, and N. Adhapure. Plant Growth Promotion Using Microbial IAA Producers in Conjunction with Azolla: A Novel Approach. *Chemical and Biological Technologies in Agriculture* 4 (2017) 1. <https://doi.org/10.1186/s40538-016-0083-3>.
- [23] R.E. Saichek, and K. R. Reddy, 2004. Evaluation of Surfactants/Cosolvents for Desorption / Solubilization of Phenanthrene in Clayey Soils. *International Journal of Environmental Studies* 61 (2004) 587–604.
- [24] S. Spaepen, and J. Vanderleyden. Auxin and Plant-Microbe Interactions. *Cold Spring Harbor Perspectives in Biology* 3 (2011) a001438–a001438. <https://doi.org/10.1101/cshperspect.a001438>.
- [25] P.P. Suryatmana, R.E. Kardena, E. Ratnaningsih, and Wisnuprpto. 2007. Improving the Effectiveness of Crude-Oil Hydrocarbon Biodegradation Employing *Azotobacter Chroococcum* as Co-Inoculant. *Microbiology Indonesia* 1 (2007) 5–10. <https://doi.org/10.5454/mi.1.1.2>.
- [26] P. Suryatmana, P., A.M. Zanatan, A, A.N. Sylvia, M.R. Setiawati, Syafrizal, Zulkifliani., A. Wulandari and A. Riztama. (2018). Bioremediation of Petroleum Contaminated Soil Using Oyster mushroom Log Waste (OMLW) *Azotobacter inelandii*, and Petrophylic consortium. *Asian Jr. of Microbiol. Biotech. Env. Sc.* 20 (2018) 2018 : S158-S168
- [27] K. Tao, X. Zhang, X. Chen, X. Liu, X. Hu, X. Yuan. Response of soil bacterial community to bioaugmentation with a plant residue-immobilized bacterial consortium for crude oil removal. *Chemosphere* 222(2019) 831–838. <http://dx.doi.org/10.1016/j.chemosphere.2019.01.133>.
- [28] J.V. Eyk. 1997. *Petroleum Bioventing*. A.A. Balkema Publishers, Netherlands, 1997.