Vol. 24, No. 2(2025) IA25552
Revista Mexicana de Ingeniería Química

Association of pyocyanin and LED red-light irradiation (700 nm) on the biodegradation of waste lube oil

Asociación de la piocianina y la irradiación de luz roja LED (700 nm) en la biodegradación de aceite lubricante usado

H. Borchardt[1], R. Leite[1], A.A.P. Mendes[1], I.P.G. Amaral[2], U. Vasconcelos[3‡]

[1] *Universidade Federal da Paraíba, Centro de Biotecnologia, Campus I, João Pessoa, Paraíba, Brasil.*

[2] *Departamento de Biologia Celular e Molecular, Centro de Biotecnologia, UFPB, Via Ipê Amarelo s/n, Campus I, CEP 58051-900, João Pessoa, Paraíba, Brasil.*

[1] *Departamento de Biotecnologia, Centro de Biotecnologia, UFPB, Via Ipê Amarelo s/n, Campus I, CEP 58051-900, João Pessoa, Paraíba, Brasil.*

Sent date: March 18, 2025; Accepted: June 20, 2025

Abstract

Billions of liters of Waste Lube Oil (WLO) are produced every year and affect the environment. *Pseudomonas aeruginosa* is a versatile bacterium that can be used in processes to remove pollutants from the environment. Its bioactive pyocyanin (PYO) is a blue pigment with multiple cellular functions including a role in the assimilation of hydrocarbons. This study assessed the association of pyocyanin and LED light irradiation (700±10 nm) in the reduction of WLO by *P. aeruginosa* TGC04. Microcosms containing 45 mL of mineral medium combined with 5% WLO (v/v) were added to 5 mL of *P. aeruginosa* TGC04, suspended in 0,9% saline solution, adjusting the turbidity to Optical Density (OD) 0.4 at 600 nm, and different concentrations of PYO (0.1; 1.0 and 10.0 µg/mL). The microcosms were incubated at 29±1°C for 30 days under continuous LED light irradiation (700±10 nm). Control was carried out in the dark. WLO reduction was calculated by gravimetric method. Abiotic losses (≈ 10%) were determined with uninoculated microcosms. Light stimulated the inoculum, reducing the WLO from 17 to 55%. The process was PYO concentration-dependent and significantly influenced by the inoculum. There was an important correlation between PYO and WLO degradation by *P. aeruginosa* TGC04. In contrast, the interactions between light irradiation and PYO, as well as light irradiation alone, were insignificant possibly due to photoinactivation. These results highlight the biotechnological applicability of *P. aeruginosa* metabolites for *ex situ* bioremediation.

Keywords: Bioremediation, Oil hydrocarbons, *Pseudomonas aeruginosa*, Bioactive pigments.

Resumen

Billones de litros de aceite lubricante usado (WLO) se producen anualmente y afectan al medio ambiente. *Pseudomonas aeruginosa* es una bacteria metabólica versátil que puede utilizarse en procesos para eliminar contaminantes del ambiente. Su metabolito bioactivo, la piocianina (PYO), es un pigmento azul con múltiples funciones celulares, incluyendo la asimilación de hidrocarburos. Este estudio evaluó la asociación de la piocianina y la irradiación con luz LED (700±10 nm) en la reducción de WLO por *P. aeruginosa* TGC04. Se añadieron microcosmos con 45 mL de medio mineral combinado con 5 % de aceite lubrificante usado (v/v) a 5 mL de una suspensión de *P. aeruginosa* TGC04 (DO600 = 0,4) y diferentes concentraciones de PYO (0,1; 1,0 y 10,0 µg/mL). Los microcosmos se incubaron a 29±1°C durante 30 días bajo irradiación continua de luz LED (700±10 nm). El control se realizó en oscuridad. La reducción de WLO se calculó mediante un método gravimétrico. Se determinaron pérdidas abióticas (≈ 10 %) con microcosmos sin inocular. La luz estimuló el inóculo y la reducción de WLO fue del 17 al 55%. El proceso fue dependiente de la concentración de PYO y estuvo significativamente influenciado por el inóculo. Se observó una correlación importante entre la degradación de PYO y WLO por *P. aeruginosa* TGC04. Por el contrario, las interacciones entre la irradiación luminosa y PYO, así como la irradiación luminosa por sí sola, fueron insignificantes posiblemente debido a la fotoinactivación. Estos resultados destacan la aplicabilidad biotecnológica de los metabolitos de *P. aeruginosa* para la biorremediación *ex situ*.

Palabras clave: biorremediacíon, hidrocarburos de petróleo, *Pseudomonas aeruginosa*, pigmentos bioactivos.

[‡] *Corresponding author. E-mail*: u.vasconcelos@cbiotec.ufpb.br;
https://doi.org/10.24275/rmiq/IA25552
ISSN:1665-2738, *issn-e*: 2395-8472

1 Introduction

Lubricant oil plays a role in reducing friction between engine parts and cooling of their components, essential for their proper functioning. During these operations, the lubricant absorbs various contaminants and is subjected to physic-chemical and thermal processes that degrade its structure, ultimately leading to oil breakdown and the formation of waste lube oil (WLO) (Raţiu *et al*., 2021). In the beginning of this decade, more than 30 billion liters were accumulated annually (Ghannam *et al*., 2021). In WLO spills, serious damage is done to the environment, affecting both terrestrial and aquatic ecosystems (Nowak *et al*., 2019). The accumulation of petroleum products in the environment can have far-reaching impacts, including high toxicity to living organisms, compromising water and soil quality, and harm to the physical, chemical, and microbiological conditions of the ecosystem, leading to long-term ecological damage (Peraza-Liñan *et al*., 2025). WLO may be also derived from chemical compounds produced from petroleum, often associated with base oils like mineral oil. Its composition includes aliphatic and aromatic hydrocarbons, such as phenol, naphthalene, benzo[a]pyrene, benzo[a]anthracene, and fluoranthene, which are significant agents in environmental contamination (Taleshpur *et al*., 2025; Ishaya *et al*., 2023; Imam *et al*., 2022).

Pseudomonas aeruginosa is widely distributed in nature and can degrade a wide range of pollutants. Even though *P. aeruginosa* is well known as an opportunistic pathogen related to nosocomial infections (Rodrigues-Recio *et al*., 2025), there are many reports on its capacity to degrade a wide range of hydrocarbons (Hu *et al*., 2023, Muthukumar *et al*., 2023; Ojewumi *et al*., 2018). Given the fact of *P. aeruginosa* dominates oil-contaminated sites, it is renowned as a keystone species in the hydrocarbon degradation processes (Centler *et al*., 2020). Additionally, the non-pathogenic nature of most strains of *P. aeruginosa* recovered from hostile environments has created industrial interest because these strains do not exhibit a serious level of risk to the environment (Jose *et al*., 2018). This has encouraged the use of *P. aeruginosa* in a wide range of biotechnological processes such as bioremediation and production of biosurfactants, biopolymers and pigments (Ruiz-Hernandes *et al*., 2024; Khan, 2022).

P. aeruginosa also synthetizes pyocyanin (PYO), a water-soluble blue phenazine with redox potential (El-Fouly al., 2015). Some studies have suggested that PYO may be useful in hydrocarbon biodegradation by *P. aeruginosa* (Das and Das, 2015; Norman *et al*., 2004). PYO acts as a signaling molecule that modulates multiple functions in the cell, resulting in hydrocarbon metabolization. PYO regulates the synthesis of rhamnolipids (Das and Ma, 2013) as response to the nutritional stress caused by the C:N ratio disbalance caused by an oil spill (Bahari *et al*., 2017). In addition, PYO as an electron acceptor reacts with molecular oxygen forming free radicals that promote nucleophilic attacks on hydrocarbons. Thus, PYO favors transformation and enhances the bioavailability of persistent hydrocarbons (Unglaube *et al*., 2020).

Good results may be achieved using bioremediation associated with abiotic methods such as ultrasound (Zhang and Zhuang, 2024) and phosphate-free mineral detergent (Saucedo-Martínez *et al*., 2023). Photodegradation enhances the kinetics and efficiency of the bioremediation process (Al-Ansari, 2021). Photolysis is an abiotic degradation of molecules either by the absorbed radiant energy of light, or by the reaction initiated by other light absorbing compounds (Kaing *et al*., 2024). Photodegradation methods mainly rely on the use of photons produced from natural sunlight or artificial lights, e.g., ultraviolet and fluorescent lamps. Absorption of radiant energy from photons can trigger physicochemical changes at the molecular level, enhancing their susceptibility to degradation (Sarker and Ahn, 2022).

Photodegradation is also the main abiotic environmental fate of hydrocarbons in nature and two mechanisms of action are addressed. First, a direct transformation based on hydrocarbons absorbs ultraviolet and visible light wavelengths (Frena *et al*., 2014). The second mechanism involves the formation of photosensitizer metabolites that react with hydrocarbons in the vicinity (Bacosa *et al*., 2015a).

By assuming that PYO absorbs light at 690 nm (Parsons *et al*., 2007), we hypothesized that Light Emitting Diode (LED) red-light irradiation could contribute to hydrocarbon degradation by triggering photolysis and increasing the hydrocarbon bioavailability to *P. aeruginosa* TGC04 because light irradiation would also cause PYO to become more reactive. Therefore, this work aimed to assess synergistic interaction of these elements in the reduction of WLO.

2 Material and Methods

2.1 *Pseudomonas aeruginosa*

The inoculum was prepared with the non-pathogenic wild-type strain *P. aeruginosa* TGC04, recovered from soil samples at a gas station in the city of João Pessoa, Brazil. The strain is registered in the Brazilian registry of genetic heritage and associated knowledge (SisGen in Portuguese, access number A404D65) and in the UFPEDA culture collection (1063B). The strain was maintained on cetrimide agar.

2.2 *Pyocyanin production*

PYO is a secondary metabolite naturally synthesized by *P. aeruginosa* strains under nutritional stress (Whooley and McLoughlin, 1982). The pre-inoculum of *P. aeruginosa* TGC04 was prepared on cetrimide agar and incubated for 48 h at 29±1°C. Afterwards, the cells from the fresh culture were suspended in 0.9% NaCl saline solution, adjusting the turbidity to Optical Density (OD) 0.4 at 600 nm (Mojsoska *et al.*, 2021). Aliquots of 5 ml of this suspension were transferred to 1000 mL flasks containing 500 ml of King A broth. These flasks were incubated at 29±1°C for 72 h. Then, the aqueous phase was mixed with chloroform (5:1 v/v) and vortexed for 1 min. The blue organic phase was separated and acidified with 0.2 mol/L HCl (2:1 v/v) and vortexed for 1 min. The red acidified phase containing the protonated PYO was neutralized by few drops of 1.5 mol/L Tris-HCl buffer solution, restoring the blue color (Peruzzo *et al.*, 2021). OD was measured at 690 nm (Kasvi, 320-1020 nm, 4NM) and PYO concentration (µg/mL) was determined by using Equation 1, obtained from a standard curve prepared by using different concentrations (0.005-0.035 mg/mL) of PYO 98% HPLC purity (sigma-Aldrich, Saint Louis, USA) hydroalcoholic solutions (1:10) with OD measured at 520 nm ($R^2 = 0.999$).

$$\text{PYO } (\mu\text{g/mL}) = (8.9968 \times \text{OD}_{690}) + 0.00004 \quad (1)$$

2.3 *Biodegradation tests*

For the biodegradation tests, there were two operational factors: light irradiation, and concentration of PYO. Two and three settings were selected, respectively, for the first and the second factor. A complete experimental design for testing all combinations of the settings and their replicates entailed 48 experiments.

Throughout the experiment, the LED red-light irradiation (700±10 nm) was continuously used. The light irradiation apparatus consisted of a closed cabin measuring 50 x 30 cm with a LED lamp (A60-E27-3-RGB-3B; 100-240 V; 60 Hz; 3 W), 13 cm distant from the microcosm lower side, exposing the system to 160.55±2.30 mW/mm^2. The control group was maintained in the dark.

Transparent polypropylene microcosms with a total capacity of 80 mL (60 × 55 mm) were filled with 45 mL of minimum mineral, composed of (g/L): K_2HPO_4 (0.5), $(NH_4)_2SO_4$ (0.5), $MgSO_4$ (0.5), $FeCl_2$ (0.01), $CaCl_2$ (0.01), $MnCl_2$ (0.0001), $ZnSO_4$ (0.0001), supplemented with 0.1 g of yeast extract and one drop of vitamin B complex solution, pH 7.2 ± 0.2 (Palittapongarnpim *et al.*, 1998). To the medium was added 2.5 mL of a mixture of WLO previously characterized (Table 1), containing 448,000 mg/Kg of the TPH.

Sixteen conditions were tested with three variables: concentration of PYO (0.1; 1.0 and 10.0 µg/mL), and with the presence or absence of LED light irradiation, as summarized in Table 2. Hydrophilic cotton pads were placed (so as not to seal) on the top of the microcosms to provide microbial respiration and avoid oil volatilization. There was no additional oxygen available to microcosms. The systems were incubated statically for 30 days at 29±1ºC. As for the control, four conditions were tested, as follows: with and without inoculum (abiotic), irradiated and unirradiated.

2.4 *Determination of hydrocarbon biodegradation*

The test was carried out using the gravimetric method (Almutairi, 2022). The mass (g) of WLO was calculated by Equation 2:

$$\% = \left(\frac{P_0 - P_1}{P_0}\right) \times 100 \quad (2)$$

Where P_0 = initial mass; and P_1 = final mass (Shimadzu, ATY224).

2.5 *Statistical analysis*

All experiments were carried out in triplicate and results are presented as the average ± standard deviation. All statistics were performed in R (version 4.2.1) in R Studio (2023.06.1 Build 524). First, the data were analyzed for normality and homoscedasticity based on the results of the Shapiro-Wilk and Levene tests respectively. Then, two-way ANOVA followed by Tukey's post-hoc test, with p-value corrected for multiple comparisons using the "false discovery rate" method was carried out. The significance value was 0.05 for all tests.

3 Results, tables and figures

The TPH content reached 448,000 mg/L and, because of the mixed nature of the WLO, the lubricant fraction represented 99.3% of the TPH. The remaining portion contained other hydrocarbon fractions, Polycyclic Aromatic Hydrocarbons (PAHs), water and impurities. Three PHAs from incomplete hydrocarbon combustion were detected, as follows: the majority of two light

Table 1. Settling Time for Hydrogen Production in Different Phases of Digestion.

Parameter	Result	Reference
Density (g/cm^3)	0.8651	ASTM D1298-12b
Total solids (%)	97.4	ASTM D874-23
TPH (mg/Kg)	448,000.0	
Gasoline (C8-C11)	< 10,226.0	
Kerosene (C11-C14)	< 10,226.0	USEPA 8015D
Diesel (C14-C20)	< 10,226.0	
Lubricating oil (C20-C40)	445,000.0	
16 EPA priority PAHs (mg/Kg)	50.1	
Naphthalene (mg/Kg)	45.3	USEPA 8270C
Pyrene (mg/Kg)	2.7	
Phenanthrene (mg/Kg)	2.1	

EPA – US environmental protection agency.

Table 2. Test conditions for biodegradation of WLO by *P. aeruginosa* TGC04

Condition	Pyocyanin(µg/mL)	Light (700±10 nm)	Inoculum (5% v/v)	Biodegradation (%)
1	0.0	+	+	32.27±0.03
2	0.0	–	+	13.34±0.09
3	0.0	+	–	9.86±0.17
4	0.0	–	–	1.79±0.10
5	0.1	+	+	51.32±0.23
6	0.1	–	+	48.00±0.11
7	0.1	+	–	17.43±0.01
8	0.1	–	–	23.94±0.01
9	1.0	+	+	44.05±0.05
10	1.0	–	+	40.12±0.15
11	1.0	+	–	19.73±0.01
12	1.0	–	–	24.53±0.01
13	10.0	+	+	55.23±0.13
14	10.0	–	+	52.40±0.11
15	10.0	+	–	21.64±0.01
16	10.0	–	–	26.08±0.01

PAHs, naphthalene and phenanthrene; and one heavy PAH pyrene (Table 1). *P. aeruginosa* TGC04 grown in the presence of WLO was positive and the strain produced 32 μg/mL of PYO. Additionally, a dense biofilm surrounding the bottom oil layer was observed. The biofilm layer was up to about twice the oily phase in height, as seen in Figure 1.

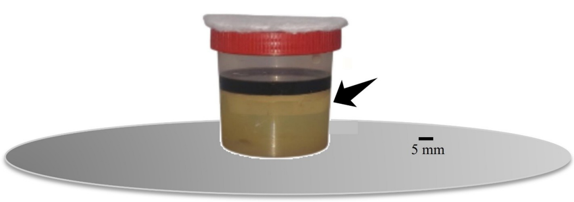

Figure 1. Appearance of the microcosm, highlighting a dense biomass at the oil phase-aqueous phase interface contributing to emulsify the WLO (arrow). The more biofilm formed, the more degradation of the WLO.

Changes in the visual appearance of the WLO were observed during the process, from a dense and dark homogeneous fluid into an emulsified light brownish product. Table 2 shows that after 30 days of incubation, there was a reduction of oil between ≈ 13 and 55% (abiotic loss ≈ 11%). Statistical analysis indicated that the main effect of inoculum size was significant ($p < 0.001$). When evaluated alone, the inoculum achieved a biodegradation rate of 13.34±0.09%. Nevertheless, under LED red-light irradiation (700±10 nm), this rate increased to 32.27±0.03%, demonstrating that the interaction between light and inoculum size was significant ($p = 0.039$). Additionally, the main effect of PYO was significant ($p < 0.001$), with a degradation between ≈ 24 and 26% when tested alone. When combined with the inoculum, however, the degradation rate increased significantly, between ≈ 48 up to 52% in the sample with a higher PYO concentration. Thus, interaction between the variables of inoculum size and pyocyanin was statistically significant when concentration-dependent (p = 0.010). In contrast, the other evaluated parameters did not yield statistically significant results, i.e., the interactions between light and PYO (p = 0.816); and the interactions between light, inoculum size, and PYO (p = 0.984); as well as the main effect of light alone (p = 0.268) (Figure 2 and Table 3).

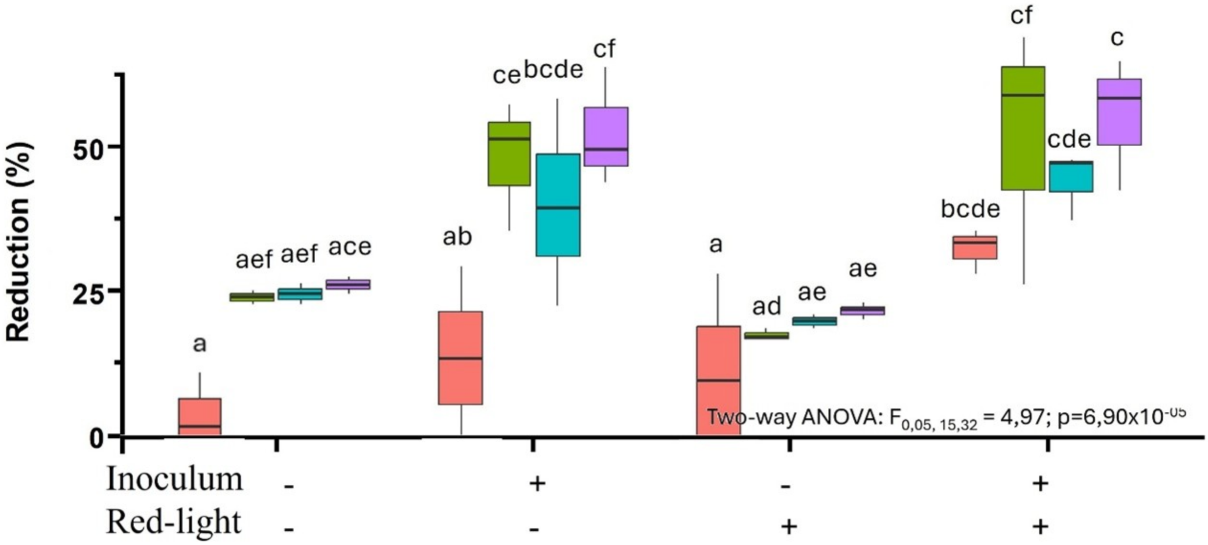

Figure 2. Reduction of WLO in relation to the initial mass after 30 days. The different colors in the graph indicate the different concentrations of pyocyanin (orange = 0.0; green = 0.1; blue = 1.0; and purple = 10.0 µg/mL) tested and the letters indicate the statistical differences obtained. Below is an indication of which test group presents inoculum and/or red-light irradiation (700±10 nm). Abiotic loss = 11.75±0.20%.

Table 3. ANOVA and estimated parameters

Factor	Sum Sq	Df	F value	*p*-value	
LT	28.04	1	1.2686	0.26841	-
Ino	2181.20	1	98.6640	$2.67x10^{-11}$	***
PYO	1211.49	3	18.2667	$4.345x10^{-7}$	***
LT:Ino	102.11	1	4.6189	0.03929	*
LT:PYO	20.72	3	0.3124	0,81627	-
Ino:PYO	292.97	3	4.4174	0,01043	*
LT:Ino:PYO	3.44	3	0.0519	0.98410	-

LT: light; Ino: Inoculum; PYO: pyocyanin 0 (***); > 0.01 (*); > 0.1 (-)

4 Discussion

WLO can cause hydrocarbon contamination; its persistent nature and its derivatives poses a severe threat to both human health and environmental safety (Benguenab and Chibani, 2021). This study aimed to evaluate how the association with a physicochemical method may be positive for the biodegradation of WLO by *P. aeruginosa* TGC04. Further, it assessed the impact of visible red-light irradiation (700±10 nm) on the reduction of this pollutant. PYO exhibits two peaks of light absorption, at ultraviolet and visible light bands (Reszka *et al.*, 2004). As the study aimed to use reactive PYO in oxidant conditions under safe wavelength irradiation, a LED lamp with an available wavelength close to 690 nm was used. We supposed that phenazine would be integrated into the process since PYO is a redox and bioactive compound important to several functions in *P. aeruginosa*, such as biofilm formation (Pierson and Pierson, 2010), a crucial factor involved in hydrocarbon biodegradation (Dasgupta *et al.*, 2013). PYO plays a role in regulating the synthesis of the biosurfactant in *P. aeruginosa*. Moreover, PYO also interacts directly with biosurfactant metabolism since it enhances the emulsification potential of *P. aeruginosa* isolates (Das and Ma, 2013). Previous studies from our group have reported that PYO participates as an inducer of molecules that emulsify different hydrocarbons even in low concentrations (Viana *et al.*, 2018). The more PYO produced by the strain, the more polycyclic aromatic hydrocarbons were reduced (Cavalcanti *et al.*, 2019). In addition, we also have observed that exogenous phenazines (PYO and phenazine methosulphate) added to encompassed inoculum of *P. aeruginosa* contributed to the removal of principally 4-6 ring PAHs (Viana *et al*., 2024).

In addition, the redox-active nature of PYO engages in electron transfer reactions, playing a pivotal role in the generation of Reactive Oxygen Species (ROS) such as superoxide and hydrogen peroxide (Saini *et al*., 2025). This could explain the significance of the variable PYO since electrochemical oxidation process increase electron transfer capacity of the organic matter. The result is an increase of WLO bioavailability as reported in previous studies with different redox compounds, e.g., humic-like substances (Deng *et al*., 2020).

Visible light-induced photocatalysis and biodegradation have been used to treat some areas (Sarkar, 2023). Light irradiation in the red spectral range promotes microbial growth (Yeh *et al*., 2014). We assumed that the application of LED red-light irradiation (700±10 nm) would be incorporated into the system as a complementary strategy to stimulate *P. aeruginosa* TGC04 to degrade WLO. This assumption was supported by the fact that variable interaction of red-light irradiation (700±10 nm) and the inoculum size have a subtle statistical significance in the biodegradation of WLO. This is further reinforced by evidence that microbes can perceive differences in light quality and can also perceive differences in light intensity with respect to red and blue light bands (Beattie *et al*., 2018). According to Crugeira *et al*. (2019), LED red-light irradiation significantly enhanced catabolic responses of a microbial consortium in their study on degradation of a textile dye. Differently from this present work, only the consortium was treated by LED red-light-irradiation.

Light may enhance biological treatment as well as abiotic transformation of persistent molecules (Sutar *et al*., 2020; Bacosa *et al*., 2015b). Sensory photoreceptors in microorganisms play a role in converting the absorbed photons into chromophore biological changes, which can include alterations in enzymatic activity or interactions with other macromolecules (Elahi and Matthew, 2024). Crugeira *et al*. (2018) reported that photostimulation by LED red-light promoted a 2.0-2.5-fold increase of cellular proliferation in a thermophilic microbial consortium due to microbial cytochrome absorption in this band. As well, there was an increase in intracellular ATP levels which activated metabolic events involving cellular proliferation. Thus, we presumed that intermittent photostimulation would support the growth of *P. aeruginosa* TGC04 influenced by red-light irradiation, as in the present study.

LED red-light irradiation (700±10 nm) acted in the degradation of the hydrocarbons present in the WLO. This was possibly because during the photocatalytic degradation process, the electrons in the valence band of photocatalysts can be excited to the conduction band and release protons (H+) after collecting photons from a light source such as the LED light used in the experiment. The electrons and protons may react with molecular oxygen and generate ROS which subsequently react with hydrocarbons and increase their bioavailability (Rayaroth *et al*., 2023; Zhang *et al*., 2022). Based on the findings of this present study we speculate that the energy emitted by the LED lamp was not capable of provoking a significant breakdown of the hydrocarbons of the WLO. In contrast, red-light LED was found suitable for the biofilm growth and both inoculum and PYO played a crucial role in the process.

On the other hand, some heavy hydrocarbons exposed to light at ≈ 700 nm may act as photosensitizers and can lead the oxidation of PYO (Reszka *et al*., 2006). This oxidation *per se* is irreversible and effectively inactivates PYO by producing 1-hydroxyphenazine (Mudaliar and Prasad, 2024). Additionally, even though a small fraction of PYO could be oxidized, the stimulation of microbiota by light irradiation compensated for the losses of PYO by inactivation and the amount of abiotic loss reinforced this claim.

In addition, in all concentrations of PYO tested, a dense layer formed by the biofilm was observed. PYO is an important factor for cell aggregation in *P. aeruginosa* (Jabłońska *et al*., 2023). Under stressful nutritional conditions, *P. aeruginosa* may promote auto-poisoning of the planktonic cells and PYO in the reduced state mediate cell lysis and release of eDNA, influencing biofilm structure and stability (Meirelles and Newman, 2018). As well, an approximately 400 µm layer with high gradient of reduced state PYO is maintained close by the upper surface within the biofilm (Koley *et al*., 2011). We hypothesized that this behavior contributed to viable cells degrading the WLO.

Despite a growing interest in the knowledge of photodegradation of organic pollutants in the natural environment, studies using specific visible-light wavelength are still limited (Guo, *et al*. 2023). The level of significance associated with the variables of inoculum size and pyocyanin have indicated their crucial role in the process, confirming that strategies based on these factors can optimize biodegradation of hydrocarbons. Despite the advances in understanding PYO as a molecule, its association with light energy and the use of *P. aeruginosa* in bioremediation remains poorly explored. Additionally, the environmental agenda required the development of sustainable approaches to solve urgent demands. This present study, for instance, calls for a possible *ex situ* application. Amounts of oily waste transferred from in-shore oil-spilled zones could be treated at industrial plants, replacing common hazardous treatments for these cases, such as incineration.

Conclusions

Under the experimental conditions carried out in this study, both variables, inoculum size and pyocyanin, alone or associated, were pivotal for optimizing the biodegradation of WLO by *P. aeruginosa* TGC04. The effect of PYO on the process was concentration-dependent under the assumption that part of PYO may be inactivated by light. In contrast, variable light irradiation (700±10 nm) exhibited some effect when combined with the inoculum and did not show a significant impact when used alone. Our findings highlight the potential for the use of microbial bioactive metabolite and renewable energy combined to provide *ex situ* bioremediation substituting treatments that negatively impact the environment. Further studies may elucidate all the mechanisms involved.

Acknowledgements

The authors want to thank to CNPq and Federal University of Paraíba for the financial support (project ID: PVI16295-2023; PIBIC/UFPB/CNPq - EDITAL 01/2023/PROPESQ). The English text of this paper has been revised by Sidney Pratt, Canadian, MAT (The Johns Hopkins University), RSAdip - TESL (Cambridge University).

Nomenclature

LED	Light Emitting Diode
OD	Optical Density
PAHs	Polycyclic Aromatic Hydrocarbons
PYO	Pyocyanin
ROS	Reactive Oxygen Species
TPH	Total Petroleum Hydrocarbons
UFPEDA	UFPE Department of Antibiotics
WLO	Waste Lube Oil

References

Al-Ansari, M. (2022). Influence of blue light on effective removal of arsenic by photosynthetic bacterium Rhodobacter sp. BT18. *Chemosphere*, *292*, 133399. https://doi.org/10.1016/j.chemosphere.2021.133399

Almutairi, M. S. (2022). Determination of total petroleum hydrocarbons (TPHs) in weathered oil contaminated soil. *Environmental Engineering Research*, *27*(5). https://doi.org/10.4491/eer.2021.324

ASTM. (2017). *D1298-12b. Standard test method for density, relative density, or API gravity of crude petroleum and liquid petroleum products by hydrometer method*. New York, USA.

ASTM. (2023). *D874-23. Standard test method for sulfated ash from lubricating oils and additives*. New York, USA.

Bacosa, H. P., Erdner, D. L., & Liu, Z. (2015a). Differentiating the roles of photooxidation and biodegradation in the weathering of Light Louisiana Sweet crude oil in surface water from the Deepwater Horizon site. *Marine Pollution Bulletin*, *95*, 265-272. https://doi.org/10.1016/j.marpolbul.2015.04.005

Bacosa, H. P., Liu, Z., & Erdner, D. L. (2015b). Natural sunlight shapes crude oil-degrading bacteria communities in northern Gulf of Mexico surface waters. *Frontiers in Microbiology*, *6*, 1325. https://doi.org/10.3389/fmicb.2015.01325

Bahari, S., Zeighami, H., Mirshahabi, H., Roudashti, S., & Haghi, F. (2017). Inhibition of Pseudomonas aeruginosa quorum sensing by subinhibitory concentrations of curcumin with gentamicin and azithromycin. *Journal of Global Antimicrobial Resistance*, *10*, 21-28. https://doi.org/10.1016/j.jgar.2017.03.006

Beattie, G. A., Hatfield, B. M., Dong, H., & McGrane, R. S. (2018). Seeing the light: The roles of red-and blue-light sensing in plant microbes. *Annual Review of Phytopathology*, *56*, 41-66. https://doi.org/10.1146/annurev-phyto-080417-045931

Benguenab, A., & Chibani, A. (2021). Biodegradation of petroleum hydrocarbons by filamentous fungi (Aspergillus ustus and Purpureocillium lilacinum) isolated from used engine oil contaminated soil. *Acta Ecologica Sinica*, *41*, 416-423. https://doi.org/10.1016/j.chnaes.2020.10.008

Cavalcanti, T. G., Souza, A. F., Ferreira, G. F., Dias, D. S. B., Severino, L. S., Morais, J. P. S., Sousa, K. A., & Vasconcelos, U. (2019). Use of agroindustrial waste in the removal of phenanthrene and pyrene by microbial consortia in soil. *Waste & Biomass Valorization*, *10*, 205-214. https://doi.org/10.1007/s12649-017-0041-8

Centler, F., Günnigmann, S., Fetzer, I., & Wendeberg, A. (2020). Keystone species and modularity in microbial hydrocarbon degradation uncovered by network analysis and association rule mining. *Microorganisms*, *8*(2), 190. https://doi.org/10.3390/microorganisms8020190

Crugeira, P. J. L., Santos, G. M. P., Oliveira, S. C. P. S., Sampaio, F. J. P., Correia, N. A., Fagnani, S. R. C. A., Chinalia, F. A., Almeida, P. F., & Pinheiro, A. L. B. (2018). Photobiological effect of Laser or LED light in a thermophilic microbial consortium. *Journal of Photochemistry and Photobiology B: Biology*, *181*, 115-121. https://doi.org/10.1016/j.jphotobiol.2018.03.006

Crugeira, P. J. L., Santos, G. M. P., Oliveira, S. C. P. S., Sampaio, F. J. P., Fagnani, S. R. C. A., Sampaio, I. C. F., Ferreira, E. S., Chinalia, F. A., & Pinheiro, A. L. B. (2019). Effects of photostimulation on the catabolic process of xenobiotics. *Journal of Photochemistry and Photobiology B: Biology*, *191*, 38-43. https://doi.org/10.1016/j.jphotobiol.2018.12.004

Das, S., & Das, P. (2015). Effects of cultivation media components on biosurfactant and pigment production from Pseudomonas aeruginosa. *Brazilian Journal of Chemical Engineering*, *32*, 317–324. https://doi.org/10.1590/0104-6632.20150322s00003262

Das, P., & Ma, L. Z. (2013). Pyocyanin pigment assisting biosurfactant-mediated hydrocarbon emulsification. *International Biodeterioration & Biodegradation*, *85*, 278-283. https://doi.org/10.1016/j.ibiod.2013.07.013

Dasgupta, D., Ghosh, R., & Sengupta, T. K. (2013). Biofilm-mediated enhanced crude oil degradation by newly isolated Pseudomonas species. *International Scholarly Research Notices*, *2013*, 250749. https://doi.org/10.5402/2013/250749

Deng, Y., Wen, J., Zhu, X., Chen, N., Feng, C., Zheng, Y., Wang, H., Chen, F., & Guo, Y. (2020). Research on the redox behavior changes of humic-like substances wastewater during electrochemical oxidation process and using the treated effluent to improve the heavily contaminated soil: Taking petroleum hydrocarbon contaminated soil as example. *Journal of Cleaner Production*, *263*, 121398. https://doi.org/10.1016/j.jclepro.2020.121398

El-Fouly, M. Z., Sharaf, A. M., Shahin, A. A. M., El-Bialy, H. A., & Omara, A. M. A. (2015). Biosynthesis of pyocyanin pigment by Pseudomonas aeruginosa. *Journal of Radiation Research and Applied Sciences*, *8*(1), 36–48. https://doi.org/10.1016/j.jrras.2014.10.007

Elahi, Y., & Baker, M. A. B. (2024). Light control in microbial systems. *International Journal of Molecular Sciences*, *25*(7), 4001. https://doi.org/10.3390/ijms25074001

Frena, M., Oliveira, C. R., Silva, C. A., Madureira, L. A. S., & Azevedo, D. A. (2014). Photochemical degradation of diesel oil in water: a comparative study of different photochemical oxidation processes and their degradation by-products. *Journal of the Brazilian Chemical Society*, *25*(7), 1372-1379. https://doi.org/10.5935/0103-5053.20140119

Ghannam, M. T., Selim, M. Y. E., Khedr, M. A. M., Bin Taleb, N. A. G., & Kaalan, N. R. (2021). Investigation of the rheological properties of waste and pure lube oil. *Fuel*, *298*, 120774. https://doi.org/10.1016/j.fuel.2021.120774

Guo, Z., Kodikara, D., Albi, L. S., Hatano, Y., Chen, G., Yoshimura, C., & Wang, J. (2023). Photodegradation of organic micropollutants in aquatic environment: Importance, factors and processes. *Water Research*, *231*, 118236. https://doi.org/10.1016/j.watres.2022.118236

Hu, F., Wang, P., Li, Y., Ling, J., Ruan, Y., Yu, J., & Zhang, L. (2023). Bioremediation of environmental organic pollutants by Pseudomonas aeruginosa: Mechanisms, methods and challenges. *Environmental Research*, *15*, 117211. https://doi.org/10.1016/j.envres.2023.117211

Imam, A., Suman, S. K., Kanaujia, P. K., & Ray, A. (2022). Biological machinery for polycyclic aromatic hydrocarbons degradation: A review. *Bioresource Technology*, *343*, 126121. https://doi.org/10.1016/j.biortech.2021.126121

Ishaya, S., Usman, S., Nweke, O. D., Adams, N. H., Umar, R., Ilyasu, N. S., Jagaba, A. H., Atangwho, I. J., & Yakasai, H. M. (2023). Degradation of used engine oil by Alcaligenes sp. strain isolated from oil contaminated site: Isolation, identification, and optimization of the growth parameters. *Case Studies in Chemical and Environmental*

Engineering, *8*, 100516. https://doi.org/10.1016/j.cscee.2023.100516

Jabłońska, J., Augustyniak, A., Dubrowska, K., & Rakoczy, R. (2023). The two faces of pyocyanin – why and how to steer its production? *World Journal of Microbiology and Biotechnology*, *39*(4), 103. https://doi.org/10.1007/s11274-023-03548-w

Jose, D., Mohandas, A., & Singh, I. S. B. (2018). A non-pathogenic environmental isolate of Pseudomonas aeruginosa MCCB 123 with biotechnological potential. *International Journal of Current Microbiology and Applied Sciences*, *7*(1), 3060-3071. https://doi.org/10.20546/ijcmas.2018.701.363

Kaing, V., Guo, Z., Sok, T., Kodikara, D., Breider, F., & Yoshimura, C. (2024). Photodegradation of biodegradable plastics in aquatic environments: Current understanding and challenges. *Science of the Total Environment*, *911*, 168539. https://doi.org/10.1016/j.scitotenv.2023.168539

Khan, N. T. (2022). Pseudomonas aeruginosa in industries. *Journal of Pharmaceutical Sciences and Drug Development*, *4*(2), 1-2. https://doi.org/10.37532/jpsdd.22.4.2.1-2

Koley, D., Ramsey, M. M., Bard, A. J., & Whiteley, M. (2011). Discovery of biofilm electrocline using real-time 3D metabolite analysis. *Proceedings of the National Academy of Sciences*, *108*(50), 19996-20001. https://doi.org/10.1073/pnas.1117298108

Meirelles, L. A., & Newman, D. K. (2018). Both toxic and beneficial effects of pyocyanin contribute to the lifecycle of Pseudomonas aeruginosa. *Molecular Microbiology*, *110*(6), 995-1010. https://doi.org/10.1111/mmi.14132

Mojsoska, B., Ghoul, M., Perron, G. G., Jenssen, H., & Alatraktchi, F. A. A. (2021). Changes in toxin production of environmental Pseudomonas aeruginosa isolates exposed to sub-inhibitory concentrations of three common antibiotics. *PLoS One*, *16*(3), e0248014. https://doi.org/10.1371/journal.pone.0248014

Mudaliar, S. B., & Prasad, A. S. B. (2024). A biomedical perspective of pyocyanin from Pseudomonas aeruginosa: its applications and challenges. *World Journal of Microbiology and Biotechnology*, *40*(3), 90. https://doi.org/10.1007/s11274-023-03889-0

Muthukumar, B., Surya, S., Sivakumar, K., Alsalhi, M. S., Rao, T. N., Devanesan, S., Arunkumar, P., & Rajasekar, A. (2023). Influence of bioaugmentation in crude oil contaminated soil by Pseudomonas species on the removal of total petroleum hydrocarbon. *Chemosphere*, *310*, 136826. https://doi.org/10.1016/j.chemosphere.2022.136826

Norman, R. S., Moeller, P., McDonald, T. J., & Morris, P. J. (2004). Effect of pyocyanin on a crude-oil-degrading microbial community. *Applied and Environmental Microbiology*, *70*(7), 4004-4011. https://doi.org/10.1128/AEM.70.7.4004-4011.2004

Nowak, P., Kucharska, K., & Kamiński, M. (2019). Ecological and health effects of lubricant oils emitted into the environment. *International Journal of Environmental Research and Public Health*, *16*(16), 3002. https://doi.org/10.3390/ijerph16163002

Ojewumi, M. E., Anenih, E. V., Taiwo, O. S., Adekeye, B. T., Awolu, O. O., & Ojewumi, E. O. (2018). A bioremediation study of raw and treated crude petroleum oil polluted soil with Aspergillus niger and Pseudomonas aeruginosa. *Journal of Ecological Engineering*, *19*(3), 226-235. https://doi.org/10.12911/22998993/83564

Palittapongarnpim, M., Pokethitiyook, P., Upatham, E. S., & Tangbanluekal, L. (1998). Biodegradation of crude oil by soil microorganisms in the tropic. *Biodegradation*, *9*(2), 83-90. https://doi.org/10.1023/a:1008272303740

Parsons, J. F., Greenhagen, B. T., Shi, K., Calabrese, K., Robinson, H., & Ladner, J. E. (2007). Structural and functional analysis of the pyocyanin biosynthetic protein PhzM from Pseudomonas aeruginosa. *Biochemistry*, *46*(7), 1821-1828. https://doi.org/10.1021/bi6024403

Peraza-Liñan, D. L., Ayíl-Gutiérrez, B. A., Urías-Salazar, A. A., López-Santillán, J. A., Estrada-Drouaillet, B., Segura-Martínez, M. T. J., & Poot-Poot, W. A. (2025). Biodegradation of crude oil by Aspergillus tubingensis: biosurfactant-producing strain. *Revista Mexicana de Ingeniería Química*, *24*(1), 1-11. https://doi.org/10.24275/rmiq/Bio24332

Peruzzo, R., Corrà, S., Costa, R., Brischigliaro, M., Varanita, T., Biasutto, L., & Szabò, I. (2021). Exploiting pyocyanin to treat mitochondrial disease due to respiratory complex III dysfunction. *Nature Communications*, *12*(1), 2103. https://doi.org/10.1038/s41467-021-22062-x

Pierson, L. S., & Pierson, E. A. (2010). Metabolism and function of phenazines in bacteria: impacts on the behavior of bacteria in the environment and biotechnological processes. *Applied Microbiology and Biotechnology*, *86*(6), 1659-1670. https://doi.org/10.1007/s00253-010-2509-3

Raţiu, S., Josan, A., Alexa, V., Cioată, V. G., & Kiss, I. (2021). Impact of contaminants on engine oil: A review. *Journal of Physics: Conference Series*, *1781*(1), 012051. https://doi.org/10.1088/1742-6596/1781/1/012051

Rayaroth, M. P., Marchel, M., & Boczkaj, G. (2023). Advanced oxidation processes for the removal of mono and polycyclic aromatic hydrocarbons–A review. *Science of The Total Environment*, *857*, 159043. https://doi.org/10.1016/j.scitotenv.2022.159043

Reszka, K. J., O'Malley, Y., McCormick, M. L., Denning, G. M., & Britigan, B. E. (2004). Oxidation of pyocyanin, a cytotoxic product from Pseudomonas aeruginosa, by microperoxidase 11 and hydrogen peroxide. *Free Radical Biology & Medicine*, *36*(11), 1448-1459. https://doi.org/10.1016/j.freeradbiomed.2004.03.011

Reszka, K. J., Denning, G. M., & Britigan, B. E. (2006). Photosensitized oxidation and inactivation of pyocyanin, a virulence factor of Pseudomonas aeruginosa. *Photochemistry and Photobiology*, *82*(2), 466-473. https://doi.org/10.1562/2005-07-29-RA-626

Rodríguez-Recio, F. R., Garza-Cervantes, J. A., Balderas-Cisneros, F. J., & Morones-Ramírez, J. R. (2025). Genomic insights into and lytic potential of native bacteriophages M8-2 and M8-3 against clinically relevant multidrug-resistant Pseudomonas aeruginosa. *Antibiotics*, *14*(2), 110. https://doi.org/10.3390/antibiotics14020110

Ruiz-Hernandez, I. H., Madrigal-Perez, L. A., & González-Hernández, J. C. (2024). The potential use of Pseudomonas aeruginosa in terrestrial and space agriculture. *Brazilian Journal of Biology*, *84*, e282664. https://doi.org/10.1590/1519-6984.282664

Saini, M., Das, S. K., Kumar, D., Dutt, G., Singh, K. K., & Prakash, D. (2025). The correlation between redox activity and antimicrobial properties of pyocyanin from Pseudomonas aeruginosa. *bioRxiv*. https://doi.org/10.1101/2025.02.17.638708

Sarkar, M. (2023). Light-responsive biodegradation of wastewater pollutants: New developments and potential perspectives. *Journal of Hazardous Materials Advances*, *10*, 100281. https://doi.org/10.1016/j.hazadv.2023.100281

Sarker, M. A. R., & Ahn, Y.-H. (2022). Green phytoextracts as natural photosensitizers in LED-based photodynamic disinfection of multidrug-resistant bacteria in wastewater effluent. *Chemosphere*, *297*, 134157. https://doi.org/10.1016/j.chemosphere.2022.134157

Saucedo-Martínez, B. C., Benavides, L. M., & Sánchez-Yañez, J. M. (2023). In the biostimulation of soil impacted by hydrocarbons the detergent emulsifies them the elimination depends on microorganisms induced by mineral solution. *Novel Aspects on Chemistry and Biochemistry*, *7*, 1-12. https://doi.org/10.9734/bpi/nacb/v7/7258A

Sutar, R. S., Barkul, R. P., Delekar, S. D., & Patil, M. K. (2020). Sunlight assisted photocatalytic degradation of organic pollutants using g-C3N4-TiO2 nanocomposites. *Arabian Journal of Chemistry*, *13*(1), 4966-4977. https://doi.org/10.1016/j.arabjc.2020.01.019

Taleshpur, S., Taghavi, L., Farahani, H. F., Rasekh, B., & Bazgir, S. (2025). Oil pollution removal using g-C3N4/PEG nanocomposite in the presence of bacterial consortium isolated from contaminated areas: optimization Study. *International Journal of Environmental Science and Technology*, *2025*, 1-8. https://doi.org/10.1007/s13762-024-06307-y

Unglaube, F., Hünemörder, P., Guo, X., Chen, Z., Wang, D., & Mejía, E. (2020). Phenazine radical cations as efficient homogeneous and heterogeneous catalysts for the crossdehydrogenative aza-henry reaction. *Helvetica Chimica Acta*, *103*(7), e2000184. https://doi.org/10.1002/hlca.202000184

USEPA. (2007). *Method 8015D. Nonhalogenated organics by gas chromatography/flame ionization detector*. Washington, USA.

USEPA. (2007). *Method 8270D. Semivolatile organic compounds by gas chromatography/mass spectrometry*. Washington, USA.

Viana, A. A. G., Borchardt, H., Dantas, J. V., Bernardes-Dias, D. S., Gurgel-Amaral, I. P., & Vasconcelos, U. (2024). Effect of exogenous phenazine addition on crude heavy oil degradation by Pseudomonas aeruginosa TGC04. *Revista Mexicana de Ingeniería Química*, *23*, 1-15. https://doi.org/10.24275/rmiq/IA24251

Viana, A. A. G., Oliveira, B. T. M., Cavalcanti, T. G., Sousa, K. A., Mendonça, E. A. M., Amaral, I. P. G., & Vasconcelos, U. (2018). Correlation between pyocyanin production and hydrocarbonoclastic activity in nine strains of Pseudomonas aeruginosa. *International Journal of Advanced Engineering Research and Science*, *5*(7), 212-223. https://doi.org/10.22161/ijaers.5.7.28

Whooley, M. A., & McLoughlin, A. J. (1982). The regulation of pyocyanin production in Pseudomonas aeruginosa. *European Journal of Applied Microbiology and Biotechnology*, *15*(3), 161-166. https://doi.org/10.1007/BF00511241

Yeh, N., Yeh, P., Shih, N., Byadgi, O., & Cheng, T. C. (2014). Applications of light-emitting diodes in researches conducted in aquatic environment. *Renewable and Sustainable Energy Reviews*, *32*, 611–618. https://doi.org/10.1016/j.rser.2014.01.047

Zhang, H., Lu, Y., Li, Y., Wang, C., Yu, Y., Zhang, W., Wang, L., Niu, L., & Zhang, C. (2022). Propelling the practical application of the intimate coupling of photocatalysis and biodegradation system: System amelioration, environmental influences and analytical strategies. *Chemosphere*, *287*, 132196. https://doi.org/10.1016/j.chemosphere.2021.132196

Zhang, Y., & Zhuang, H. (2024). Effectiveness of low-intensity ultrasound on anaerobic bioaugmentation of phenolic wastewater: Model optimization and system stabilization. *Bioresource Technology*, *406*,30980.https://doi.org/10.1016/j.biortech.2024.130980