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## Summary of the role of pyocyanin in the transformation and biodegradation of Polycyclic Aromatic Hydrocarbons

### Resumo sobre o papel da piocianina na transformação e biodegradação de Hidrocarbonetos Policíclicos Aromáticos

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#### ABSTRACT

Pyocyanin is an active redox phenazine of intense blue color and specific to *Pseudomonas aeruginosa*. The synthesis of the molecule confers different benefits to the bacterium. Pyocyanin can control its growth and persistence in environments with high nutritional pressures, forming biofilms. As well, synthesis of pyocyanin enables *P. aeruginosa* tolerate and uptake highly toxic compounds such as polycyclic aromatic hydrocarbons (PAHs), considered the most dangerous compounds among all molecules found in crude oil and petroderivatives. Additionally, pyocyanin increases the bioavailability of PAHs and its metabolites are used to synthesize crucial molecules for the biodegradation of other PAHs. On the other hand, oil hydrocarbons can serve as oxygen vectors during the synthesis of pyocyanin, contributing to the sustainability of the biodegradation process. This review is a compilation of recent advances reported in the literature about the relationship between pyocyanin expression and the hydrocarbonoclastic activity of *P. aeruginosa*. This characteristic, for the pyocyanin-deficient strains, is important for the degradation of PAHs, a topic that has been unevenly studied.

**Keywords:** Biodegradation; *Pseudomonas aeruginosa*; Quorum sensor; Redox active compounds.

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#### RESUMO

A piocianina é uma fenazina redox ativa de cor azul intensa, específica da *Pseudomonas aeruginosa* e a síntese da molécula confere diferentes benefícios à bactéria. A piocianina pode controlar seu crescimento e persistência em ambientes com altas pressões nutricionais, formando biofilmes. Além disso, a síntese de piocianina permite que a bactéria tolere e absorva compostos altamente tóxicos, como os hidrocarbonetos aromáticos policíclicos (HPA), considerados os compostos mais perigosos entre todas as moléculas presentes no petróleo bruto e petroderivados. Além disso, a piocianina aumenta a biodisponibilidade dos HPA e seus metabólitos, usados para sintetizar moléculas cruciais para a biodegradação de outros HPA. Por outro lado, hidrocarbonetos de petróleo podem servir como vetores de oxigênio durante a síntese de piocianina, contribuindo para a sustentabilidade do processo de biodegradação. Esta revisão é uma compilação dos avanços recentes relatados na literatura sobre a relação entre a expressão de piocianina e a atividade hidrocarbonoclástica de *P. aeruginosa*. Essa característica nas linhagens piocianina-deficientes é importante para a degradação de HPA, um tema ainda pouco estudado.

**Palavras-chave:** Biodegradação; Compostos redox ativos; Quorum sensor; *Pseudomonas aeruginosa*.

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## INTRODUCTION

Commercial oil exploration has established a strong dependence on human activity throughout its modern history, promoting significant benefits to society from the application of this raw material to various sectors of industry (BLACK, 2020). The major collateral effects of this dependence, however, have been their environmental repercussions along the entire oil chain (KHARAKA et al., 2005). Because petroleum comprises a complex mixture of organic compounds with a predominance of hydrocarbons, impacts on the environment are inevitable, especially caused by the most recalcitrant molecules (VARJANI, 2017). Polycyclic Aromatic Hydrocarbons (PAHs) are recalcitrant because their stability in the chemical structure helps them to adsorb easily into different matrices (UKALSKA-JARUGA et al., 2020). As well, their absorbance by plant and animal cells causes disturbances in the food chain, and may lead to mutagenesis and cancer (QUADEER et al., 2019; SANTOS et al., 2014).

Given all these negative health impacts of PAHs, ecofriendly strategies to remove them is welcome (NAEEM; QAZI, 2020). The use of hydrocarbonoclastic microbes offers the possibility of total clean up employing various sustainable techniques (SANGWAN; DUKARE, 2018). The ability of bacteria to exhibit a diverse catalytic metabolic capacity has been known since the early 20<sup>th</sup> century, with research showing these microbes to be suitable agents for biodegradation (mineralization) and biotransformation (modification of organic compounds) of PAHs and other oil hydrocarbons (SILVA et al., 2021).

This paper highlights the potential for cleaning up PAHs from the environment by *Pseudomonas aeruginosa*, a ubiquitous rod-shaped bacterium acknowledged for its remarkable metabolic ability to enhance the natural biodegradation of various xenobiotic pollutants (LIU et al., 2022; PALLERONI, 2010). *P. aeruginosa* is also a species that easily adapts to hostile environments with strongly limiting factors, including those with organic matter difficult to uptake. The sense of limiting nutritional conditions leads *P. aeruginosa* to differentiation, forming subpopulations of metabolically inactive cells as a survival strategy in PAHs-contaminated sediments (CIOFU; TOLKER-NIELSEN, 2019). The catabolite repressor control protein is involved in this strategy (ZHANG et al., 2012), but part of these mechanisms can also be attributed to pyocyanin synthesis (VANDRISSE et al., 2021). The pyocyanin molecule is specific to *P. aeruginosa*, and it is synthesized by a significant number of the total strains (MAVRODI et al., 2001). As a

redox-active molecule, pyocyanin can contribute to an increase in the bioavailability of various organic compounds (ASHOUR et al., 2021). In addition, pyocyanin is a signaling molecule that regulates gene expression in response to fluctuations in cell-population density (READING; SPERANDIO, 2006), important in the process of biodegradation of toxic compounds.

There is a lack of understanding in our knowledge about the correlation of the role of pyocyanin to the hydrocarbonoclastic activity of *P. aeruginosa*; the little material available in the literature, however, provides some ideas and suggests some areas of research that may be investigated to understand better the ecological niche of the bacterium.

## **WHAT WE KNOW ABOUT PAHs AND MICROBIAL DENSITY**

PAHs are hydrocarbons whose chemical structure is formed by two or more aromatic rings or condensed cyclopentanes, making these molecules chemically stable, with low water solubility and high partition coefficients (POATER et al., 2018; MANZETTI, 2013). PAHs are highly lipophilic, therefore, easily absorbed by animals (ALEGBELEYE et al., 2017) and accumulated in plants (YAKOVLEVA et al., 2016). Additionally, PAHs spontaneously adsorb to sediment and organic matter, occurring in aggregates in soil (GALGANI et al., 2011) and water (BELLES et al., 2016).

PAHs are formed from incomplete combustion of organic substances (WANG et al. 2017) or through *de novo* reactions (PENG et al., 2018); however, more than 90% of the PAHs in the environment originate from human activity (HARITASH; KAUSHIK, 2009). According to the United States Environmental Protection Agency (USEPA), sixteen types of PAHs are most prevalent in the environment (Table 1). Because they vary in degrees of toxicity, mutagenicity, and carcinogenicity, they are recognized as research priorities (RAVINDRA et al., 2008). The USEPA has been considering increasing the number of priority PAHs up to 26 because their occurrence, bioavailability and toxicity are still uncertain and deserve attention. These emerging molecules are known as non-USEPA priority PAHs (GAO et al., 2019).

**Table 1** – 16 priority PAHs and some of their properties

PAHs	Rings	Carcinogenicity*	Solubility (mg.L <sup>-1</sup> )	Log K <sub>w/o</sub>
Naphthalene	2	2B	31.0	3.4
Acenaphthene	3	3	3.8	3.9
Acenaphthylene	3	---	16.1	4.1
Fluorene	3	3	1.9	4.2
Phenanthrene	3	3	1.1	4.6
Anthracene	3	3	4.5x10 <sup>-2</sup>	4.5
Fluoranthene	4	3	2.6x10 <sup>-1</sup>	5.2
Pyrene	4	3	13.2x10 <sup>-2</sup>	5.2
Benzo[a]anthracene	4	2B	1.1x10 <sup>-2</sup>	5.6
Chrysene	4	2B	1.5x10 <sup>-3</sup>	5.9
Benzo[b]fluoranthene	5	2B	1.5x10 <sup>-3</sup>	6.1
Benzo[k]fluoranthene	5	2B	8.0x10 <sup>-4</sup>	6.8
Benzo[a]pyrene	5	1	3.8x10 <sup>-3</sup>	6.5
Dibenzo[ah]anthracene	5	2B	5.0x10 <sup>-3</sup>	6.5
Benzo[ghi]perylene	6	3	2.6x10 <sup>-4</sup>	6.6
Indeno[1,2,3-cd]pyrene	6	2B	6.2x10 <sup>-2</sup>	7.1

\*carcinogenic for humans according 2022 latest update IARC groups: 1 – carcinogenic; 2A – probably carcinogenic; 2B – possibly carcinogenic; 3 – not classifiable as to its carcinogenicity to humans). Data collected from Bojes and Pope (2007) and Cai et al. (2007).

The 16 priority PAHs are classified according to the number of aromatic rings. Low molecular weight ones have two to three rings, while high molecular weight ones have between four and six rings (KIM et al., 2013). Because of the considerable complexity of the chemical structure of high molecular weight PAHs, they exhibit lower solubility in water (RABONI; VIOTTI, 2016), as well as show more lipophilicity, compared to low-molecular PAHs (SVERDRUP et al., 2002).

When introduced into a given ecosystem, PAHs can disturb the food chain. The way these contaminants are exposed to organisms and trophic positions of these organisms in the food web, however, may influence the diffusion of PAHs in the environment (ASHOK et al., 2022). Microbes comprise the second level of the food web of any system (STEFFAN et al., 2015). The presence of PAHs can lead to a natural microbial community that can reach inhibition as high as 70% (LABUD et al., 2007), because of the accumulation of toxic substances (DEMANÈCHE et al., 2004), thus resulting in reduction of catalytic metabolism (BOUCHEZ et al., 1995).

Subsequently, the dominant biomass develops into a composition of hydrocarbonoclastic microorganisms (BENEDEK et al., 2013; TERAMOTO et al., 2013). The hydrocarbonoclastic microbiota is also autochthonous; before the introduction of the contaminant, however, its number is quite low compared to other species, and can

increase by up to 1000 times after the introduction of hydrocarbons (JONES et al., 2020). In addition, this variation in the dynamics of the microbial community favors the biotransformation of the contaminant, since the cometabolic relationships act as a mechanism to produce intermediate metabolites necessary for the restoration of microbial population, in density and diversity, corresponding to the amount prior to contamination (JOHNSEN et al. al., 2005).

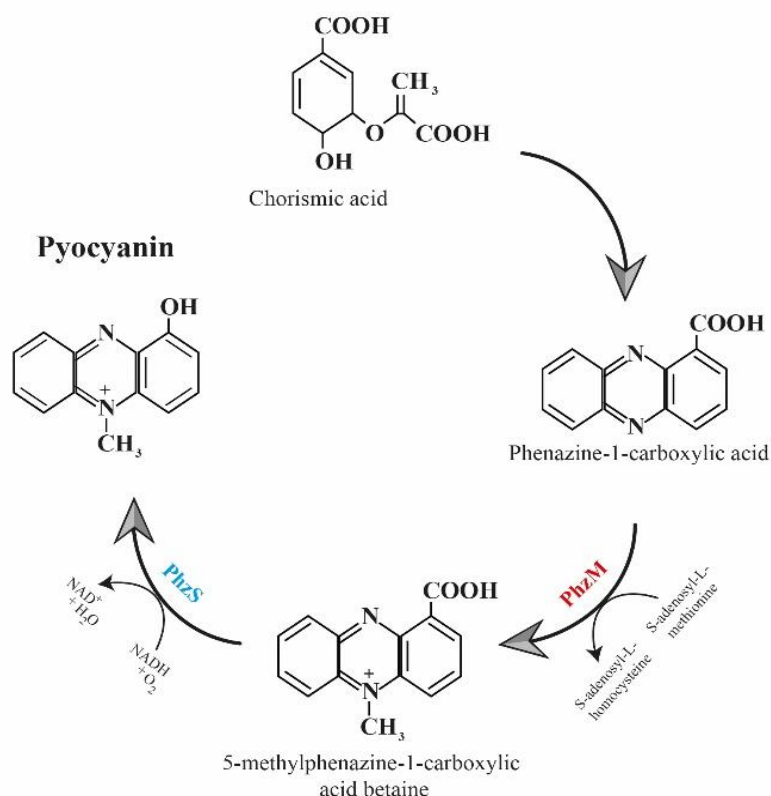
### ***Pseudomonas aeruginosa* AND ITS LUXURIANT BLUE PIGMENT**

*P. aeruginosa* is a motile rod-shaped aerobic Gram-negative bacterium that possesses a remarkable metabolic capacity, which confers the advantage of being widespread in diverse environments, such as soil, fresh water, plants and animals (VIANA et al., 2017). This metabolic capacity also gives *P. aeruginosa* the possibility of using more than 90 organic compounds as sources of carbon and energy (FRIMMERSDORF et al., 2010; SCOTT-THOMAZ, 2010), including PAHs and other hydrocarbons (LI et al., 2021; LONG et al., 2019; JACQUES et al., 2005). This characteristic enables *P. aeruginosa* to be effective in the processes of removing oil hydrocarbons from different matrices (VARJANI et al., 2020; DWIVEDI et al., 2011).

Although there are differences in genome size in terms of the origin of strains, whether wild, clinical, or industrial (WEISER et al., 2019), *P. aeruginosa* strains synthesize different metabolites involved in the processes that contribute to *P. aeruginosa*'s permanence in hostile environments, such as nutrient-limiting ones (ARRUDA et al., 2019). Edaphic microbes commonly synthesize phenazines as a mechanism linked to competition for exhibiting antibiosis activity (BIESSY; FILION, 2018). Phenazines are nitrogen-heterocyclic core compounds that induce many physiological effects on *P. aeruginosa* as well as species in association with the bacterium (PIERSON III; PIERSON, 2010; JO et al., 2020).

Pyocyanin (5-methyl-1-hydroxyphenazine) is the most important phenazine produced by *P. aeruginosa*. The molecule is a deep blue pigment, common in 90 to 95% of the strains (OLIVEIRA et al., 2019). The blue molecule is composed of two subunits of N-methyl-1-hydroxyphenazine synthesized by a pathway associated with chorismate (Figure 1), controlled by seven genes involving two operons (JAYASEELAN et al., 2014).

**Figure 1** – Graphical summary of the steps in the synthesis of pyocyanin



Pyocyanin is a redox-active compound associated with the formation of reactive oxygen species (ROS) (BAHARI et al., 2017). As well, it acts as a quorum sensor (LIU; NIZET, 2009) and electron acceptor, enabling glucose oxidation and subsequent ATP formation (GLASSER et al., 2014). These mechanisms can indirectly or directly favor the detoxification processes of pollutants, as will be discussed later. Additionally, in pyocyanin-producing strains, hydrocarbon degradation is more effective (NORMAN et al., 2004), which reinforces the role of the pigment in the hydrocarbonoclastic activity of *P. aeruginosa*.

## HOW PYOCYANIN MAY BE INVOLVED IN THE TRANSFORMATION OF PAHs

The microbial transformation of organic matter involves numerous cell-cell relationships, whose interactions ensure synchronism in the colonization and communication between individuals of the same or related species, aiming at the removal of organic carbon and its reintroduction into the food web (URVOY et al., 2022). For quorum-dependent microbial interactions to occur, low-molecular-weight diffusible molecules, called autoinducers, are produced and secreted by bacteria and serve as signals

of gene expression within the producing species, as well as between different and ecologically associated species (FEDERLE; BASSLER, 2003).

Multiple molecules of N-acyl-homoserine lactones (AHL), which are produced from a minimum of 500 cells, are the main autoinducer in the two well-defined quorum sensing (QS) systems in *P. aeruginosa*, *las* and *rhs* systems (GONÇALVES; VASCONCELOS, 2021), also acting on events associated with hydrocarbon removal. Thus, it is believed that AHL-based modulation can play a pivotal role in the biodegradation of hydrocarbons by *P. aeruginosa* (HUANG et al., 2013). In addition, there is evidence that pyocyanin also acts indirectly as an autoinducer in the degradation of hydrocarbons. It is obvious that pyocyanin does not have a surfactant property, but there are strong indications that the molecule serves as a signaling molecule involved in the bases of *P. aeruginosa*'s hydrocarbonoclastic activity (DAS; DAS, 2015), as described in the following items.

## BIOSURFACTANT SYNTHESIS

Biosurfactants are molecules with surface-active properties, produced by numerous microorganisms (MOSHTAG et al., 2021). They solubilize hydrophobic substrates, allowing microbial access to these compounds (MNIF; GRHIBI, 2015). Mono- and di-rhamnolipids are the best-known glycolipid biosurfactants, largely produced by *P. aeruginosa* (KASKATEPE; YILDIZ, 2016); these molecules are fundamental for the degradation of PAHs (BEZZA; CHIRWA, 2016). The reduction of the surface tension of hydrocarbons is proportional to the degradation rate, achieving more than 90% degradation in axenic cultures and even higher when *P. aeruginosa* grows in consortia (MISHRA et al., 2014).

There are reports on the correlation of pyocyanin production with the emulsification index of some petroderivatives. In 2013, two strains of *P. aeruginosa* were compared. The first strain produced twice as much pyocyanin as produced by the second strain, resulting in a 10-fold increase in the emulsification index of three petroderivatives, whose values ranged between 60 and 75% (DAS; MA, 2013). In 2018, a second independent study identified a significant correlation (92.6%) ( $p=0.07$ ) between pyocyanin synthesis and the emulsification index of kerosene and lubricating oil in nine wild-type *P. aeruginosa* isolates. One of these strains, TGC02, produced about 28  $\mu\text{g/mL}$



of pyocyanin and the lubricating oil emulsification index was determined at 100% (VIANA et al., 2018).

## BIOFILM FORMATION

The preferred lifestyle of *P. aeruginosa* is on biofilms (EMERENINI et al., 2015). These communities are cellular organizations formed by a complex mechanism modulated by genes and operons, involving cellular structures, QS, and cellular signaling, among others (NEVES et al., 2021).

The biofilm confers protection, tolerance, and resistance to *P. aeruginosa* against toxic molecules, including PAHs (MANGWANI et al., 2016), and plays a role in the transformation of these molecules both in the aquatic (OMAROVA et al., 2019) and terrestrial environments (LOBO et al., 2002). This information may serve as a basis for the use of the biofilm-mediated bioremediation technique in place of using planktonic microorganisms (MELIANI; BENSOLTANE, 2014).

Pyocyanin participates in various mechanisms during the cycle of a biofilm, such as quorum sensor (YAN; WU, 2019), phenotype expression (GUPTE et al., 2021) and induces production of eDNA (AMLY et al., 2021). The pyocyanin-eDNA complex interferes with the hydrophobicity of the cell surface and creates conditions for colonization and development of robust biofilms (DAS et al., 2016). In most cases, however, it is poorly adhered to substrates (DELIGIANNI et al., 2010). This characteristic, attributed to the evolution and adaptation of *P. aeruginosa* facilitates detachment and avoids competition for nutrients and space with other hydrocarbonoclastic bacteria (AMER et al., 2015).

## ENZYMATIC ACTIVITY

Mono-oxygenases and dioxygenases are the main oxidoreductases that participate in the microbial degradation of oil and derivatives (ARORA et al., 2009). *P. aeruginosa* can express different genes that enable the bacteria to grow in crude oil using it as a carbon source. *alkB* and *alkB*-related genes that encode monooxygenases can participate in the conversion of long-chain saturated alkanes (BELHAJ et al., 2002), while C12O, C23O and PAH-RHD $\alpha$  genes encode dioxygenases (MUKHERJEE et al., 2017). In particular, the PAH-RHD $\alpha$  gene has an important role in the initial step of hydrocarbon degradation because the gene encodes enzymes that incorporate molecular oxygen into the nucleus of



aromatic hydrocarbons, contributing to molecular destabilization (XUE et al., 2021; CÉBRON et al., 2008).

PAHs are enzymatically transformed through the action of mono- and dioxygenases, leading to the formation of intermediates from the central metabolism, which are converted by other oxygenases to form catechol (CHEBBI et al., 2017). Subsequently, the breakage of the catechol ring can lead to the synthesis of citrate, succinate and fumarate, among other citric acid cycle intermediates (ZHANG et al., 2006). In addition, a recent study identified a complex gene network employed by *P. aeruginosa* in the degradation of PAHs. Forty-six genes related to the degradation of PAHs were analyzed and a heterogeneity in the gene networks and in the regrouping of genes was observed under different conditions. Thus, the authors hypothesized that there were six different aspects. This involved, for example the classification of genes and understanding of their mechanisms, to shed light on the role of gene interactions and reorganizations on cells exposed to environmental stresses (YAN; WU, 2017).

In addition, enzyme production also depends on QS (KARIMINIK et al., 2017). As previously mentioned, QS systems participate in the formation of the biofilm in *P. aeruginosa*. Similarly, catabolic genes and biofilm formation are related in bioremediation, since gene expression can increase with increasing concentration of PAHs in the medium (KUMARI et al., 2020) within a certain limit, as when expression gene becomes deregulated. This fact was also reported with other bacteria with increased expression of dioxygenases (MUTHUKAMALAM et al., 2017).

## **HYDROCARBONS MAY CONTRIBUTE TO PYOCYANIN SYNTHESIS**

Pyocyanin is synthesized in basal concentrations, starting at the end of the exponential phase and during the stationary phase (AGRAWAL; CHAUHAN, 2016). Environmental stresses, in particular nutritional deficiency, induce *P. aeruginosa* to synthesize the pigment; this serves as a competitive survival tool (RASHID; ANDLEEB, 2018). The variation in the concentration of  $\text{PO}_4^{3+}$  and  $\text{Ca}^{+2}$  ions is a critical factor under these conditions (WHOOLEY; McLOUGHLIN, 1982). Additionally, phosphate is crucial in regulating the production of secondary metabolites; *P. aeruginosa* is subtly sensitive to changes in ion concentration. Low phosphate values cause energy reduction and pyocyanin can act as an intracellular regulator of ATP in starvation situations (GONÇALVES et al., 2021).

As stated earlier, the presence of PAHs in the environment is a factor that limits the growth of many microbes. As a *P. aeruginosa* strategy, redox cycling contributes to creation of biomass, as well as the maintenance of redox homeostasis in oxygen-limited environments (PRICE-WHELAN et al., 2007). Thus, some hydrocarbons, such as n-hexadecane and n-hexane, can serve as oxygen vectors, which stimulate and increase pyocyanin synthesis (OZDAL et al., 2019). Oxygen is the principal component for the conversion of the intermediate phenazine, 5-methylphenazine-1-carboxylic acid betaine to pyocyanin, a reaction mediated by a flavin-dependent monooxygenase (PhzS) (JIMENEZ et al., 2012). Furthermore, because the pigment is a competitive respiratory factor and quorum sensor of *P. aeruginosa* (MORKUNAS et al., 2012), the increase in pyocyanin concentration benefits the subsequent processes involved in the biotransformation and biodegradation of hydrocarbons, such as biofilm formation and synthesis of surfactants, reaffirming the ecological importance of the bacterium.

### **PYOCYANIN INCREASES THE BIOAVAILABILITY OF PAHs**

As already mentioned, pyocyanin is a redox-active phenazine. The molecule can be reduced by NADPH and NADH (LAURSEN; NIELSEN, 2004). Thus, the reactions of one or two reduced pyocyanin intermediate electrons with molecular oxygen generate ROS, particularly superoxide, that participates in the production of hydrogen peroxide ( $H_2O_2$ ) and the hydroxyl radical ( $OH\bullet$ ) (SINHA et al., 2015; BRITIGAN et al., 1992). There is an intrinsic dependence on the availability of  $H_2O_2$  to produce  $OH\bullet$  (BABUPONNUSAMI; MUTHUKUMAR, 2014). In addition, the hydroxyl radical has a high oxidizing potential ( $E^\circ = 2.8V$ ), which enables it to attack many organic compounds (KAHOUSH et al., 2018).

Thus, ROS produced through the redox activity of pyocyanin may favor the increase in the bioavailability of chemically stable hydrocarbons, such as PAHs, through abiotic degradation. This process not only leads to the transformation of the contaminant, but also benefits the mineralization of PAHs, via microbial metabolism (NIE et al., 2020). In addition, the attack of ROS reduces the stability of PAHs, as well as increases the polarity of these compounds (MA et al., 2006). Consequently, the molecules become more bioavailable and potentially assimilable, and can later be converted into biomass and metabolites (SILVA et al., 2021).

## PYOCYANIN AS A BIOREMEDIATION STRATEGY

Fenton oxidation is a detoxification strategy for oil-contaminated sites because it can completely oxidize these contaminants (LIU et al., 2019). The treatment is based on a redox reaction based on the breakdown of  $\text{H}_2\text{O}_2$ , catalyzed by iron and other transition metals (Me), into hydroxyl and/or hydroxyl radical (Eq. 1), important to the oxidation of different organic contaminants (TALVENMÄKI et al., 2021; FRIEDRICH et al., 2017).



Pyocyanin can be reduced by NADPH and NADH to form ROS; this can be applied in the transformation of organic contaminants as a bioremediation method (GU et al., 2016). Due to the recognition and acceptance of bioremediation in terms of causing lower environmental impacts compared to other techniques (PATEL et al., 2022), *P. aeruginosa* can be associated with bioremediation when applied to the bio-Fenton technique (RAFAQAT et al., 2022). The technique is based on the generation of  $\text{H}_2\text{O}_2$ , catalyzed by enzymatic reaction (KAHOUSH et al., 2018). This significantly reduces costs, since industrial production of hydrogen peroxide is expensive (OSEGUEDA et al., 2012).

The bio-Fenton technique associated with bioremediation is very effective in detoxifying aromatic compounds because it serves as a pre-oxidation step of the contaminant (VALDERRAMA et al., 2009; LEE; HOSOMI, 2001). Pre-oxidation causes the contaminant to be more soluble in water and consequently increases its bioavailability (KULIK et al., 2006; NAM et al., 2001). It has been suggested, however, that the action of pyocyanin on bio-Fenton technique has an oxidative characteristic, without the need for additional treatment, reinforcing its relevance in the degradation of PAHs (NIE et al., 2020). This technique may produce fewer negative environmental impacts, such as the loss of fertility in soils, as are seen in chemical treatments (LAURENT et al., 2012).

## CAN INTERMEDIATE PHENAZINES CONTRIBUTE TO THE HYDROCARBONOCLASTIC ACTIVITY OF *P. aeruginosa*?

Pyocyanin is beneficial for *P. aeruginosa* since phenazines play an important role in the bacterial life cycle (MEIRELLES; NEWMAN, 2018). The conversion of phenazine-1-carboxylic acid (PCA) to pyocyanin occurs via an extrametabolic pathway

that contains both *phzM* and *phzS* enzymes. While the overexpression of *PhzM* contributes to greater production of pyocyanin (WANG et al., 2020), up to 15% of *P. aeruginosa* strains may not produce the pigment, even under laboratory conditions (GOVAN, 2012).

Part of this can be attributed to a  $\Delta$ *phzM* deficiency (MULLER; MERRETT, 2014) or mutations in important genes such as *phnAB* and *phzB1* (LAU et al., 2004). As a result, restriction from social cheating (CASTAÑEDA-TAMEZ et al., 2018) to reduction of virulence (ALLEN et al., 2005) may be observed. However, previous studies have suggested that defective pyocyanin producing mutants may exhibit competitive advantages or virulence because they develop compensatory mechanisms, such as extracellular production of the *Pseudomonas* quinolone signal (PQS) or simply because pyocyanin does not act as the only active redox metabolite (CHIEDA et al., 2007).

The intermediate phenazine PCA serves as an electron acceptor for ATP production in the absence of pyocyanin. *P. aeruginosa* produces at least six phenazines, which have been correlated with amination and sulfonation (GLASSER et al., 2017). In addition, the correlation of the activity of these other phenazines with the degradation of hydrocarbons may contribute to an understanding of the mechanisms developed by strains of pyocyanin-deficient *P. aeruginosa* to maintain their hydrocarbonoclastic activity. Despite being reduced, in comparison to pyocyanin producing strains, hydrocarbonoclastic activity remains present and effective (NORMAN et al., 2004).

In addition, it is known that pyocyanin and intermediate phenazines produced by *P. aeruginosa* can serve as a source of carbon for certain microbes, however little is known about how pyocyanin concentration is modulated under *in situ* conditions and how this may affect fitness of members of a distinct community (COSTA et al., 2015). This illustrates, however, the crucial role of *P. aeruginosa* in the maintenance of microsystems, suggesting that the bacterium may assume a strategic role as a keystone species by coexisting with other hydrocarbonoclastic microbes in the same community.

## CONCLUSION

PAHs can alter the dynamics of ecosystem services due to their structural complexity and low solubility. Consequently, an application of sustainable techniques in the remediation of these contaminants is required. The hydrocarbonoclastic activity of *P. aeruginosa* linked to its mechanisms of tolerance to PAHs has been well reported.

However, the action of pyocyanin in the degradation of these pollutants has been little explored and requires an expansion of investigations, especially on the role of pyocyanin in the process and how the pigment may be used in bioprocesses for detoxifying oil-contaminated sites.

We suggest that pyocyanin can be crucial both in biodegradation and transformation of PAHs through different mechanisms that may involve cell signaling. By this, oxidoreductases and biosurfactants are synthesized and biofilms are formed, up to the point where the bioavailability of PAHs is increased by the active redox property of pyocyanin. In addition, hydrocarbons can serve as oxygen vectors and influence pyocyanin synthesis. The pigment can serve as well as a carbon source for biomass formation in hydrocarbonoclastic communities. On the other hand, hydrocarbonoclastic activity in pyocyanin-deficient *P. aeruginosa* strains is also possible; we emphasize, however, that research on the participation of intermediate phenazines in the degradation of PAHs should inspire and encourage further investigations highlighted by this document.

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## CONFLICTS OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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## REFERENCES

AGRAWAL, A.H.; CHAUHAN, P.B. Effect of cultivation media components on pyocyanin production and its application in antimicrobial property. **Int J Curr Adv Res.** v. 5, n. 4, p. 829–833, 2016.

ALEGBELEYE, O.O.; OPEOLU, B.O.; JACKSON, V.A. Polycyclic aromatic hydrocarbons: a critical review of environmental occurrence and bioremediation. **Environ Manag.** v. 60, n. 4, p. 758-783, 2017.

ALLEN, L.; DOCKRELL, D.H.; PATTERY, T.; LEE, D.G.; CORNELIS, P.; HELLEWELL, P.G.; WHYTE, M.K.B. Pyocyanin production by *Pseudomonas aeruginosa* induces neutrophil apoptosis and impairs neutrophil-mediated host defenses *in vivo*. **J Immunol.** v. 174, n. 6, p. 3643-3649, 2005. doi: 10.4049/jimmunol.174.6.3643.

AMER, R.A.; MAPELLI, F.; EL GENDI, H.M.; BARBATO, M.; GODA, D.A.; CORSINI, A.; CAVALCA, L.; FUSI, M.; BORIN, S.; DAFFONCHIO, D.; ABDEL-FATTAH, Y.R. Bacterial diversity and bioremediation potential of the highly contaminated marine sediments at El-Max District (Egypt, Mediterranean sea). **BioMed Res Int.** v. 2015, p. 981829, 2015. doi: 10.1155/2015/981829.

AMLY, D.A.; HAJARDHINI, P.; JONARTA, A.L.; YULIANTO, H.D.K.; SUSILOWATI, H. Enhancement of pyocyanin production by subinhibitory concentration of royal jelly in *Pseudomonas aeruginosa*. **F1000 Res.** v. 10, p. 14, 2021. doi: 10.12688/f1000research.27915.4.

ARORA, P.K.; KUMAR, M.; CHAUHAN, A.; RAGHAVA, G.P.S.; JAIN, R.K. OxDBase: a database of oxygenases involved in biodegradation. **BMC Res Notes.** v. 2, p. 67, 2009. doi: 10.1186/1756-0500-2-67.

ARRUDA, R.R.A.; OLIVEIRA, B.T.M.; BONIFÁCIO, T.T.C.; MORAIS, V.C.; AMARAL, I.P.G.; VASCONCELOS, U. Activity of two exometabolites produced by *Escherichia coli* on the synthesis of pyocyanin. **Int J Adv Eng Res Sci.** v. 6, p. 267–271, 2019.

ASHOK, A.; HØJ, L.; BRINKMAN, D.L.; NEGRI, A.P.; AGUSTI, S. Food-chain length determines the level of phenanthrene bioaccumulation in corals. **Environ Pollut.** v. 297, p. 118789, 2022. doi: /10.1016/j.envpol.2022.118789.

ASHOUR, E.A.; FARSI, R.M.; ALAIDAROOS, B.A.; ABDEL-MONEIM, A-M. E.; EL-SAADONY, M.T.; OSMAN, A.O.; SAYED-AHMED, E.T.A.; ALBAQAMI, N.M.; SHAFI, M.E.; TAHA, A.E.; EL-HACK, M.E.A. Impacts of dietary supplementation of pyocyanin powder on growth performance, carcass traits, blood chemistry, meat quality and gut microbial activity of broilers. **Ital J Anim Sci.** v. 20, n. 1, p. 1357–1372, 2021. doi: 10.1080/1828051X.2021.1924087.

BABUPONNUSAMI, A.; MUTHUKUMAR, K. A review on Fenton and improvements to the Fenton process for wastewater treatment. **J Environ Chem Eng.** v. 2, n. 1, p. 557-572, 2014.

BAHARI, S.; ZEIGHAMI, H.; MIRSHAHABI, H.; ROUDASHTI, S.; HAGHI, F. Inhibition of *Pseudomonas aeruginosa* quorum sensing by subinhibitory concentrations of curcumin with gentamicin and azithromycin. **J Glob Antimicrob Resist.** v. 10, p. 21-28, 2017.

BELHAJ, A.; DESNOUES, N.; ELMERICH, C. Alkane biodegradation in *Pseudomonas aeruginosa* strains isolated from a polluted zone: identification of *alkB* and *alkB*-related genes. **Res Microbiol.** v. 153, n. 6, p. 339–344, 2002. doi:10.1016/s0923-2508(02)01333-5.

BELLES, A.; ALARY, C.; MAMINDY-PAJANY, Y.; ABRIAK, N.E. Relationship between the water-exchangeable fraction of PAH and the organic matter composition of sediments. **Environ Pollut.** v. 219, p. 512-518, 2016.

BENEDEK, T.; VAJNA, B.; TÁNCICS, A.; MÁRIALIGETI, K.; LÁNYI, S.; MÁTHÉ, I. Remarkable impact of PAHs and TPHs on the richness and diversity of bacterial species in surface soils exposed to long-term hydrocarbon pollution. **World J Microbiol Biotechnol.** v. 29, n. 11, p. 1989-2002, 2013.

BEZZA, F.A.; CHIRWA, E.M.N. Biosurfactant-enhanced bioremediation of aged polycyclic aromatic hydrocarbons (PAHs) in creosote contaminated soil. **Chemosphere.** v. 144, p. 635-644, 2016.

BIESSY, A.; FILION, M. Phenazines in plant-beneficial *Pseudomonas* spp.: Biosynthesis, regulation, function and genomics. **Environ Microbiol.** v. 20, n. 11, p. 3905-3917, 2018.

BLACK, B.C. **Crude reality: Petroleum in world history (exploring world history).** 2<sup>nd</sup> ed. Lanham-MA: Rowman & Littlefield Publishers, 304p, 2020.

BOJES, H.K.; POPE, P.G. Characterization of EPA's 16 priority pollutant polycyclic aromatic hydrocarbons (PAHs) in tank bottom solids and associated contaminated soils at an exploration and production sites in Texas. **Regul Toxicol Pharmacol.** v. 47, n. 3, p. 288-295, 2007.

BOUCHEZ, M. ; BLANCHET, D. ; VANDECASTEELE, J.P. Degradation of polycyclic aromatic hydrocarbons by pure strains and by defined strain associations: inhibition phenomena and cometabolism. **Appl Microbiol Biotechnol.** v. 43, n. 1, p. 156-164, 1995.

BRITIGAN, B.E.; ROEDER, T.L.; RASMUSSEN, G.T.; SHASBY, D.M.; McCORMICK, M.L.; COX CD. Interaction of the *Pseudomonas aeruginosa* secretory products pyocyanin and pyochelin generates hydroxyl radical and causes synergistic damage to endothelial cells: Implications for *Pseudomonas*-associated tissue injury. **J Clin Invest.** v. 90, n. 6, p. 2187-2196, 1992.

CAI, Q-Y. ; MO, C-H. ; WU, Q-T. ; ZENG, Q-Y. ; KATSOYIANNIS, A. Bioremediation of polycyclic aromatic hydrocarbons contaminated sewage sludge by different composting process. **J Harzard Mater.** v. 142, n. 1-2, p. 535-542, 2007.

CASTAÑEDA-TAMEZ, P.; RAMÍREZ-PERIS, J.; PÉREZ-VELÁZQUEZ, J.; KUTTLER, C.; JALALIMANESH, A.; SAUCEDO-MORA, M.A.; JIMÉNEZ-CORTÉS, J.G.; MAEDA, T.; GONZÁLEZ, Y.; TOMÁS, M.; WOOD, T.K.; GARCÍA-CONTRERAS, R. Pyocyanin restricts social cheating in *Pseudomonas aeruginosa*. **Front Microbiol.** v. 9, p. 01348, 2018. doi: 10.3389/fmicb.2018.01348.



CEBRON, A. ; NORINI, M-P. ; BEGUIRISTAIN, T. ; LEYVAL, C. Real-time PCR quantification of PAH-ring hydroxylating dioxygenase (PAH-RHD $\alpha$ ) genes from Gram positive and Gram negative bacteria in soil and sediment samples. **J Microb Methods**. v. 73, n. 2, p. 148-159, 2008.

CHEBBI, A.; ENTATI, D.; ZAGHDEN, H.; BACCAR, N.; REZGUI, F.; CHALBI, M.; CHAMKHA, M. Polycyclic aromatic hydrocarbon degradation and biosurfactant production by a newly isolated *Pseudomonas* sp. strain from used motor oil-contaminated soil. **Int Biodeter Biodegrad**. v. 122, p. 128-140, 2017.

CHIEDA, Y.; IIYAMA, K.; LEE, J.M.; KUSAKABE, T.; YASUNAGA-AOKI, C.; SHIMIZU, S. Inactivation of pyocyanin synthesis genes has no effect on the virulence of *Pseudomonas aeruginosa* PAO1 toward the silkworm, *Bombyx mori mori*. **FEMS Microbiol Lett**. v. 278, p. 101-107, 2007.

CIOFU, O.; TOLKER-NIELSEN, T. Tolerance and resistance of *Pseudomonas aeruginosa* biofilms to antimicrobial agents—how *P. aeruginosa* can escape antibiotics. **Front Microbiol**. v. 3, n. 10, p. 913, 2019. doi: 10.3389/fmicb.2019.00913.

COSTA, K.C.; BERGKESSEL, M.; SAUNDERS, S.; KORLACH, J.; NEWMAN, D.K. Enzymatic degradation of phenazines can generate energy and protect sensitive organisms from toxicity. **mBio**. v. 6, n. 6, p. e01520-15, doi: 10.1128/mBio.01520-15, 2015.

DAS, S.; DAS P. Effects of cultivation media components on biosurfactant and pigment production from *Pseudomonas aeruginosa*. **Braz J Chem Eng**. v. 32, n. 2, p. 317–324, 2015.

DAS, T.; IBUGO, A.I.; KLARE, W.; MANEFIELD, M. Role of pyocyanin and extracellular DNA in facilitating *Pseudomonas aeruginosa* biofilm formation. In: DHANASEKARAN, D.; THAJUDDIN, N. (Ed.). **Microbial biofilms – importance and applications**, London: ItechOpen, p. 23-42, 2016. doi: 10.5772/63497.

DAS, P.; MA L.Z. Pyocyanin pigment assisting biosurfactant-mediated hydrocarbon emulsification. **Int Biodegrad Biodeterior**. v. 85, p. 278-283, 2013. doi: 10.1016/j.ibiod.2013.07.013.

DELIGIANNI, E.; PATTISON, S.; BERRAR, D.; TERNAN, N.G.; HAYLOCK, R.W.; MOORE, J.E.; ELBORN, S.J.; DOOLEY, J.S.G. *Pseudomonas aeruginosa* cystic fibrosis isolates of similar RAPD genotype exhibit diversity in biofilm forming ability *in vitro*. **BMC Microbiol**. v. 10, p. 38, 2010. doi: 10.1186/1471-2180-10-38.

DEMANÈCHE, S. ; MEYER, C. ; MICOUD, J. ; LOUWAGIE, M. ; WILLISON, J.C. ; JOUANNEAU, Y. Identification and functional analysis of two aromatic-ring-hydroxylating dioxygenases from a *Sphingomonas* strain that degrades various polycyclic aromatic hydrocarbons. **Appl Environ Microbiol**. v. 70, n. 11, p. 6714-6725, 2004.

DWIVEDI, S.; SINGH, B.R.; AL-KHEDHAIRY, A.A.; MUSARRAT, J. Biodegradation of isoproturon using a novel *Pseudomonas aeruginosa* strain JS-11 as a multi-functional bioinoculant of environmental significance. **J Hazard Mater.** v. 185, n. 2-3, p. 938-944, 2011.

EMERENINI, B.O.; HENSE, B.A.; KUTTLER, C.; EBERL, H.J. A mathematical model of quorum sensing induced biofilm detachment. **PLOS One.** v. 10, p. e0132385, 2015. doi: 10.1371/journal.pone.0132385.

FEDERLE, M.J.; BASSLER, B.L. Interspecies communication in bacteria. **J Clin Invest.** v. 112, n. 9, p. 1291-1299, 2003.

FRIEDRICH, L.C.; ZANTA, C.L.P.; MACHULEK, A.; QUINA, F.H. Mechanistic study of the fenton and cupro-fenton reactions by voltammetric analysis *in situ*. **Quím Nova.** v. 40, n. 7, p. 769-773, 2017.

FRIMMERSDORF, E.; HORATZEK, S.; PELNIKEVICH, A.; WIEHLMANN, L.; SCHOMBURG, D. How *Pseudomonas aeruginosa* adapts to various environments: a metabolomic approach. **Environ Microbiol.** v. 12, n. 6, p. 1734-1747, 2010.

GALGANI, F.; ELLERBRAKE, K.; FRIES, E.; GOREUX, C. Marine pollution: let us not forget beach sand. **Environ Sci Europe.** v. 23, n. 1, p. 40, 2011. doi: 10.1186/2190-4715-23-40.

GAO, P.; DA SILVA, E.B.; TOWNSEND, T.; LIU, X.; MA, L.Q. Emerging PAHs in urban soils: Concentrations, bioaccessibility, and spatial distribution. **Sci Total Environ.** v. 670, n. 6, p. 800-805, 2019.

GLASSER, N.R.; KERN, S.E.; NEWMAN, D.K. Phenazine redox cycling enhances anaerobic survival in *Pseudomonas aeruginosa* by facilitating generation of ATP and a protonmotive force. **Molec Microbiol.** v. 92, p. 399- 412, 2014.

GLASSER, N.R.; WANG, B.X.; HOY, J.A.; NEWMAN, D.K. The Pyruvate and  $\alpha$ -ketoglutarate dehydrogenase complexes of *Pseudomonas aeruginosa* catalyze pyocyanin and phenazine-1-carboxylic acid reduction via the subunit dihydrolipoamide dehydrogenase. **J Biol Chem.** v. 292, n. 13, p. 5593-5607, 2017. doi: 10.1074/jbc.M116.772848.

GONÇALVES, T.; OLIVEIRA, B.T.M.; VASCONCELOS, U. Uso de piocianina no tingimento de fibra de algodão. **Int J Develop Res.** v. 11, n. 2, p. 44127-44134, 2021.

GONÇALVES, T.; VASCONCELOS, U. Colour me blue: the history and the biotechnological potential of pyocyanin. **Molecules.** v. 26, p. 927, 2021. doi: 10.3390/molecules26040927.

GOVAN, J.R.W. Pseudomonads and non-fermenters: Opportunist infection; cystic fibrosis; melioidosis. In: GREENWOOD, D.; SLACK, R.C.B.; BARER, M.R.; IRVING, W.L. (Ed.) **Medical Microbiology.** London: Churchill livingstone, p. 298–304, 2012, doi:10.1016/B978-0-7020-4089-4.00043-3.

GU, C.; WANG, J.; LIU, S.; LIU, G.; LU, H.; JIN, R. Biogenic Fenton-like reaction involvement in cometabolic degradation of tetrabromobisphenol A by *Pseudomonas* sp. **Environ Sci Technol**. v. 50, n. 18, p. 9981–9989, 2016.

GUPTE, A.; JYOT, J.; RAVI, M.; RAMPHAL, R. High pyocyanin production and non-motility of *Pseudomonas aeruginosa* isolates are correlated with septic shock or death in bacteremic patients. **PlosOne**. v. 16, n. 6, p. e0253259, 2021. doi:10.1371/journal.pone.0253259.

HARITASH, A.K.; KAUSHIK, C.P. Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. **J hazard Mater**. v. 169, n. 1-3, p. 1-15, 2009.

HUANG, Y. ; ZENG, Y. ; YU, Z. ; ZHANG, J. ; FENG, H. ; LIN, X. *In silico* and experimental methods revealed highly diverse bacteria with quorum sensing and aromatics biodegradation systems - A potential broad application on bioremediation. **Bioresour Technol**. v. 148, p. 311–316, 2013. doi:10.1016/j.biotech.2013.08.155.

IARC - INTERNATIONAL AGENCY FOR RESEARCH ON CANCER. IARC monographs on the evaluation of carcinogenic risks to humans. Available at: <<http://monographs.iarc.fr/ENG/Classification/index.php>>, access 26 Jun 2022.

JACQUES, R.J.S.; SANTOS, E.C.; BENTO, F.M.; PERALBA, M.C.R.; SELBACHA, P.A.; SÁ, L.S.S.; CAMARGO, F.A.O. Anthracene biodegradation by *Pseudomonas* sp. isolated from a petrochemical sludge landfarming site. **Int Biodegrad Biodeterior**. v. 56, n. 3, p. 143-150, 2005.

JAYASEELAN, S.; RAMASWAMY, D.; DHARMARAJ, S. Pyocyanin: production, applications, challenges and new insights. **World J Microbiol Biotechnol**. v. 30, n. 4, p. 1159-1168, 2014.

JIMENES, P.N.; KOCK, G.; THOMPSON, J.A.; XAVIER, K.B.; COOL, R.H.; QUAX, W.J. The multiple signaling systems regulating virulence in *Pseudomonas aeruginosa*. **Microbiol Molec Biol Rev**. v. 76, n. 1, p. 46-65, 2012.

JO, J.; PRICE-WHELAN, A.; CORNELL, W.C.; DIETRICH, L.E. Interdependency of respiratory metabolism and phenazine-associated physiology in *Pseudomonas aeruginosa* PA14. **J Bacteriol**. v. 202, n. 4, p. e00700-19, 2020. doi: 10.1128/JB.00700-19.

JOHNSEN, A.R.; WICK, L.Y.; HARMS, H. Principles of microbial PAH-degradation in soil. **Environ Pollut**. v. 133, n. 1, p. 71-84, 2005.

JONES, A.M.; JAMES, I.I.; AKPAN, P.S.; EKA, I.I.; ORUK, A.E.; IBUOT, A.A. Characterization of hydrocarbon utilizing bacteria in waste engine oil-impacted sites. **BioRxiv**. v. 2020, p. 998872, 2020. doi: 10.1101/2020.03.21.998872.

KAHOUSH, M.; BEHARY, N.; CAYLA, A.; NIERSTRASZ, V. Bio-Fenton and Bio-electro-Fenton as sustainable methods for degrading organic pollutants in wastewater. **Process Biochem**. v. 64, p. 237-247, 2018.

KARIMINIK, A.; BASERI-SALEHI, M.; KHEIRKHAN, B. *Pseudomonas aeruginosa* quorum sensing modulates immune responses: An updated review article. **Immunol Lett.** v. 190, p. 1-6, 2017. doi: 10.1016/j.imlet.2017.07.002.

KASKATEP, B.; YILDIZ, S. Rhamnolipid biosurfactants produced by *Pseudomonas* species. **Braz Arch Biol Technol.** v. 59, p. e16160786, 2016. doi: 10.1590/1678-4324-2016160786.

KHARAKA, Y.K.; THORSEN, J.J.; KAKOUROS, E.; HERKELRATH, W.N. Impacts of petroleum production on ground and surface waters: Results from the Osage–Skiatook Petroleum Environmental Research A site, Osage County, Oklahoma. **Environ Geosci.** v. 12, n. 2, p. 127-138, 2005.

KIM, K.; JAHAN, S.A.; KABIR, E.; BROWN, R.J. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. **Environ Int.** v. 60, p. 71-80, 2013.

KULIK, N.; GOI, A.; TRAPIDO, M.; TUHKANEN, T. Degradation of polycyclic aromatic hydrocarbons by combined chemical pre-oxidation and bioremediation in creosote contaminated soil. **J Environ Manag.** v. 78, n. 4, p. 382-391, 2006.

KUMARI, S.; MANGWANI, N.; DAS, S. Naphthalene catabolism by biofilm forming marine bacterium *Pseudomonas aeruginosa* N6P6 and the role of quorum sensing in regulation of dioxygenase gene. **J Appl Microbiol.** v. 130, n. 4, p. 1217-1231, 2020.

LABUD, V.; GARCIA, C.; HERNANDEZ, T. Effect of hydrocarbon pollution on the microbial properties of a sandy and a clay soil. **Chemosphere.** v. 66, n. 10, p. 1863-1871, 2007.

LAU, G.W.; RAN, H.; KONG, F.; HASSETT, D.J.; MAVRODI, D. *Pseudomonas aeruginosa* pyocyanin is critical for lung infection in mice. **Infect Immun.** v. 72, n. 7, p. 4275–4278, 2004.

LAURENT, F.; CÉBRON, A.; SCHWARTZ, C.; LEYVAL, C. Oxidation of a PAH polluted soil using modified Fenton reaction in unsaturated condition affects biological and physico-chemical properties. **Chemosphere.** v. 86, n. 6, p. 659-664, 2012.

LAURSEN, J.B.; NIELSEN, J. Phenazine natural products: biosynthesis, synthetic analogues, and biological activity. **Chem Rev.** v. 104, n. 3, p. 1663-1686, 2004.

LEE, B.; HOSOMI, M. A hybrid Fenton oxidation–microbial treatment for soil highly contaminated with benzo(a)anthracene. **Chemosphere.** v. 43, n. 8, p. 1127-1132, 2001.

LI, J.; CHEN, W.; ZHOU, W.; WANG, Y.; DENG, M.; ZHOU, S. Synergistic degradation of pyrene by *Pseudomonas aeruginosa* PA06 and *Achromobacter* sp. AC15 with sodium citrate as the co-metabolic carbon source. **Ecotoxicology.** v. 30, p. 1487–1498, 2021. doi: 0.1007/s10646-020-02268-3.

LIU, G.Y.; NIZET, V. Color me bad: Microbial pigments as virulence factors. **Trends Microbiol.** v. 17, p. 406-413, 2009.

LIU, H.; YANG, G.; JIA, H.; SUN, B. Crude oil degradation by a novel strain *Pseudomonas aeruginosa* AQNU-1 isolated from an oil-contaminated lake wetland. **Processes**. v. 10, n. 2, p. 307, 2022. doi:10.3390/pr10020307.

LIU, M-H.; HSIAO, C-H.; WANG, Y-S.; CHEN, W-Y.; HUNG, J-M. Tandem modified Fenton oxidation and bioremediation to degrade lubricant-contaminated soil. **Int Biodegrad Biodeterior**. v. 143, p. 104738, 2019. doi: 10.1016/j.ibiod.2019.104738.

LOBO, C.; SANCHEZ, M.; GARBI, C.; FERRER, E.; MARTINEZ-IÑIGO, M.J.; ALLENDE, J.L.; MARTÍN, C.; CASASÚS, L.; ALONSO, R.; GIBELLO, A.; MARTIN M. Immobilized native bacteria as a tool for bioremediation of soils and waters: implementation and modeling. **Sci World J**. v. 18, n. 2, p. 1361-1368, 2002.

LONG, M.; WEN, L.; MUTAI, B.; PEIYAN, S. Effect of surfactants on the solubilization, sorption and biodegradation of benzo (a) pyrene by *Pseudomonas aeruginosa* BT-1. **J Taiwan Inst Chem Eng**. v. 96, p. 121-130, 2019.

MA, J. ; MA, W. ; SONG, W. ; CHEN, C. ; TANG, Y. ; ZHAO, J. ; ZHANG, L. Fenton degradation of organic pollutants in the presence of low-molecular-weight organic acids: Cooperative effect of quinone and visible light. **Environ Sci Technol**. v. 40, n. 2, p. 618-624, 2006.

MANGWANI, N.; SHUKLA, S.K.; KUMARI, S.; DAS, S.; RAO, T.S. Effect of biofilm parameters and extracellular polymeric substance composition on polycyclic aromatic hydrocarbon degradation. **RSC Adv**. v. 6, n. 62, p. 57540–57551, 2016.

MANZETTI, S. Polycyclic aromatic hydrocarbons in the environment: environmental fate and transformation. **Polycycl Aromat Compd**. v. 33, n. 4, p. 311-330, 2013.

MAVRODI, D.V.; BONSALL, R.F.; DELANEY, S.M.; SOULE, M.J.; PHILLIPS, G.; THOMASHOW, L.S. Functional analysis of genes for biosynthesis of pyocyanin and phenazine-1-carboxamide from *Pseudomonas aeruginosa* PAO1. **J Bacteriol**. v. 183, n. 21, p. 6454-6465, 2001.

MEIRELLES, L.A.; NEWMAN, D.K. Both toxic and beneficial effects of pyocyanin contribute to the lifecycle of *Pseudomonas aeruginosa*. **Mol Microbiol**. v. 110, n. 6, p. 95-101, 2018.

MELIANI, A.; BENSOLTANE, A. Enhancement of hydrocarbons degradation by use of *Pseudomonas* biosurfactants and biofilms. **J Pet Environ Biotechnol**. v. 5, n. 1, p. 1000168, 2014. doi: 10.4172/2157-7463.1000168.

MULLER, M.; MERRETT, N.D. Pyocyanin production by *Pseudomonas aeruginosa* confers resistance to ionic silver. **Antimicrob Agents Chemother**. v. 58, n. 9, p. 5492–5499, 2014.

MISHRA, S.; SINGH, S.N.; PANDE, V. Bacteria induced degradation of fluoranthene in minimal salt medium mediated by catabolic enzymes *in vitro* condition. **Bioresour Technol**. v. 164, p. 299-308, 2014.

MNIF, I.; GHRIBI, D. Review lipopeptides biosurfactants: mean classes and new insights for industrial, biomedical, and environmental applications. **J Pept Sci.** v. 104, n. 3, p. 129-147, 2015.

MORKUNAS, B.; GALLOWAY, W.R.J.D.; WRIGHT, M.; IBBESON, B.M.; HODGKINSON, J.T.; O'CONNELL, K.M.G.; BARTOLUCCI, N.; DELLA VALLE, M.; WELCH, M.; SPRING, D.R. Inhibition of the production of the *Pseudomonas aeruginosa* virulence factor pyocyanin in wild-type cells by quorum sensing autoinducer-mimics. **Org Biomol Chem.** v. 10, p. 8452-8464, 2012.

MOSHTAGH, B.; HAWBOLDT, K.; ZHANG, B. Biosurfactant production by native marine bacteria (*Acinetobacter calcoaceticus* P1-1A) using waste carbon sources: Impact of process conditions. **Can J Chem Eng.** v. 99, n. 11, p. 2386-2397, 2021.

MUKHERJEE, A.K.; BHAGOWATI, P.; BISWA, B.B.; CHANDA, A.; KALITA, B. A comparative intracellular proteomic profiling of *Pseudomonas aeruginosa* strain ASP-53 grown on pyrene or glucose as sole source of carbon and identification of some key enzymes of pyrene biodegradation pathway. **J Proteom.** v. 167, n. 1, p. 25-35, 2017.

MUTHUKAMALAM, S.; SIVAGANGAVATHI, S.; DHRISHYA, D.; RANI, S.S. Characterization of dioxygenases and biosurfactants produced by crude oil degrading soil bacteria. **Braz J Microbiol.** v. 48, p. 637-647, 2017.

NAEEM, U.; QAZI, M.A. Leading edges in bioremediation technologies for removal of petroleum hydrocarbons. **Environ Sci Pollut Res.** v. 27, n. 22, p. 27370-27382, 2020.

NAM, K.; RODRIGUEZ, W.; KUKOR, J.J. Enhanced degradation of polycyclic aromatic hydrocarbons by biodegradation combined with a modified Fenton reaction. **Chemosphere.** v. 45, n. 1, p. 11-20, 2001.

NEVES, M.L.R.; NUNES, L.E.; ROCHA, W.R.V.; XIMENES, E.C.P.A.; ALBUQUERQUE, M.C.P.A. The influence of *quorum sensing* on the formation of biofilm by *Pseudomonas aeruginosa*. **Res Soc Develop.** v. 10, n. 2, p. e33910212659, 2021. doi: 10.33448/rsd-v10i2.12659.

NIE, H.; NIE, M.; DIWU, Z.; WANG, L.; QIAO, Q.; ZHANG, B.; YANG, X. Homogeneously catalytic oxidation of phenanthrene by the reaction of extracellular secretions of pyocyanin and Nicotinamide Adenine Dinucleotide. **Environ Res.** v. 191, p. 110159, 2020. doi: 10.1016/j.envres.2020.110159.

NORMAN, R.S.; MOELLER, P.; McDONALD, T.J.; MORRIS, P.J. Effect of pyocyanin on a crude-oil-degrading microbial community. **Appl Environ Microbiol.** v. 70, n. 7, p. 4004-4011, 2004.

OLIVEIRA, B.T.M.; BARBOSA, P.S.Z.; CAVALCANTI, T.G.; AMARAL, I.P.G.; VASCONCELOS, U. Craft beer waste as substrate for pyocyanin synthesis. **J Pharm Biol Sci.** v. 14, n. 1, p. 21-25, 2019.



- OMAROVA, M.; SWIENTONIEWSKI, L.T.; TSENGAM, I.K.M.; BLAKE, D.A.; JOHN, V.; McCORMICK, A.; BOTHUN, G.D.; RAGHAVAN, S.R.; BOSE, A. Biofilm formation by hydrocarbon-degrading marine bacteria and its effects on oil dispersion. **ACS Sustain Chem Eng**. v. 7, n. 17, p. 14490–14499, 2019.
- OSEGUEDA, O.; DAFINOV, A.; LLORCA, J.; MEDINA, F.; SUERIAS, J. *In situ* generation of hydrogen peroxide in catalytic membrane reactors. **Catal Today**. v. 193, n. 1, p. 128-136, 2012.
- OZDAL, M.; GURKOK, S.; OZDAL, O.G. Enhancement of pyocyanin production by *Pseudomonas aeruginosa* via the addition of n-hexane as an oxygen vector. **Biocatal Agric Biotechnol**. v. 22, p. 101365, 2019. doi: 10.1016/j.bcab.2019.101365.
- PALLERONI, N.J. The *Pseudomonas* story. **Environ Microbiol**. v. 12, n. 6, p. 1377-13783, 2010. doi: 10.1111/j.1462-2920.2009.02041.x.
- PATEL, A.K.; SINGHANIA, R.; ALBARICO, F.P.J.; PANDEY, A.; CHEN, C-W.; DONG, C-D. Organic wastes bioremediation and its changing prospects. **Sci Total Environ**. v. 824, p. 153889, 2022. doi: 10.1016/j.scitotenv.2022.153889.
- PENG, Y. ; BUEKENS, A. ; TANG, M. ; LU, S. Mechanochemical treatment of fly ash and *de novo* testing of milled fly ash. **Environ Sci Pollut Res**. v. 25, n. 19, p. 19092-19100, 2018.
- PIERSON III, L.S. ; PIERSON, E.A. Metabolism and function of phenazines in bacteria: Impacts on the behavior of bacteria in the environment and biotechnological processes. **Appl Microbiol Biotechnol**. v. 86, n. 6, p. 1659-1670, 2010.
- POATER, J.; DURAN, M.; SOLÀ, M. Aromaticity determines the relative stability of kinked vs. straight topologies in polycyclic aromatic hydrocarbons. **Front Chem**. v. 20, n. 6, p. 561, 2018. doi: 10.3389/fchem.2018.0056.
- PRICE-WHELAN, A.; DIETRICH, L.E.P.; NEWMAN, D.K. Pyocyanin alters redox homeostasis and carbon flux through central metabolic pathways in *Pseudomonas aeruginosa* PA14. **J Bacteriol**. v. 189: 6372–6381, 2007.
- QADEER, A.; HOU, L.; YANG, J.; LI, X.; KHALIL, S.K.; HUANG Y, AL MAMUM MH, GAO D, YANG Y. Trophodynamics and parabolic behaviors of polycyclic aromatic hydrocarbons in an urbanized lake food web, Shanghai. **Ecotoxicol Environ Saf**. v. 178, p. 17-24, 2019. doi: 10.1016/j.ecoenv.2019.04.003.
- RABONI, M.; VIOTTI, P. 2016. Formation and destruction of Polycyclic Aromatic Hydrocarbons (PAHs) in the flaring of the biogas collected from an automotive shredded residues landfill. **Rev Ambient Água**. v. 11, n. 1, p. 4-12, 2016.
- RAFAQAT, S.; ALI, N.; TORRES, C.; RITTMANN, B. Recent progress in treatment of dyes wastewater using microbial-electro-Fenton technology. **RSC Adv**. v. 12, p. 17104-17137, 2022.



RASHID, M.I.; ANDLEEB, S. **Pyocyanin yield improvement for enhancement of *Pseudomonas aeruginosa* inoculated Microbial Fuel Cell efficiency**. 2018 International Conference on Power Generation Systems and Renewable Energy Technologies (PGSRET). doi:10.1109/pgsret.2018.8685940.

RAVINDRA, K.; SOKHI, R.; Van GRIEKEN R. Atmospheric polycyclic aromatic hydrocarbons: source attribution, emission factors and regulation. **Atmos Environ**. v. 42, n. 13, p. 2895-2921, 2008.

READING, N.C.; SPERANDIO, V. Quorum sensing: the many languages of bacteria. **FEMS Microbiol Lett**. v. 254, n. 1, p. 1-11, 2006. doi: 10.1111/j.1574-6968.2005.0001.x.

SANGWAN, S.; DUKARE, A. Microbe-mediated bioremediation: an eco-friendly sustainable approach for environmental clean-up. In: ADHYA, T.K.; LAL, B.; MOHAPATRA, B.; PAUL, D.; DAS, S. (Ed.). **Advances in soil microbiology: recent trends and future prospects. microorganisms for sustainability**. Springer Natyre: Singapore, 2018, p. 145-163.

SANTOS, R.G.; LOH, W.; BANNWART, A.C.; TREVISAN, O.V. An overview of heavy oil properties and its recovery and transportation methods. **Braz J Chem Eng**. v. 31, n. 3, p. 571-590, 2014.

SCOTT-THOMAS, A.; SYHRE, M.; PATTEMORE, P.K.; EPTON, M.; LAING, R.; PEARSON, J.; CHAMBERS, S.T. 2-Aminoacetophenone as a potential breath biomarker for *Pseudomonas aeruginosa* in the cystic fibrosis lung. **BMC Pulm Med**. v. 10, n. 1, p. 1-10, 2010.

SILVA, E.S.; PRAGANA, L.G.; VASCONCELOS, U. Photooxidation vs biodegradation: A short review on fate of heavy hydrocarbons after oil spill in sea water. **Int J Eng Res Appl**. v. 11, n. 5, p. 8-17, 2021.

SINHA, S.; SHEN, X.; GALLAZZI, F.; LI, Q.; ZMIJEWSKI, J.W.; LANCASTER, J.R. Jr.; GATES, K.S. Generation of reactive oxygen species mediated by 1-hydroxyphenazine, a virulence factor of *Pseudomonas aeruginosa*. **Chem Res Toxicol**. v. 28, n. 2, p. 175-181, 2015.

STEFFAN, S.A.; CHIKARAISHI, Y.; CURRIE, C.R.; HORN, H.; GAINES-DAY, H.R.; PAULI, J.N.; ZALAPA, J.E.; OHKOUCHI, N. Microbes are trophic analogs of animals. **PNAS**. v. 112, n. 49, p. 15119-15124, 2015. doi: 10.1073/pnas.1508782112.

SVERDRUP, L.E.; NIELSEN, T.; KROGH, P.H. Soil ecotoxicity of polycyclic aromatic hydrocarbons in relation to soil sorption, lipophilicity, and water solubility. **Environ Sci Technol**. v. 36, n. 11, p. 2429-2435, 2002.

TALVENMÄKI, H.; SAARTAMA, N.; HAUKKA, A.; LEPIKKÖ, K.; PAJUNEN, V.; PUNKARI, M.; GUOYONG, Y.; SINKKONEN, A.; PIEPPONEN, T.; SILVENNOINEN, H.; ROMANTSCHUK, M. *In situ* bioremediation of Fenton's reaction-treated oil spill site, with a soil inoculum, slow release additives, and methyl- $\beta$ -cyclodextrin. **Environ Sci Pollut Res.** v. 28, p. 20273–20289, 2021. doi: 10.1007/s11356-020-11910-w.

TERAMOTO, M.; QUECK, S.Y.; OHNISHI, K. Specialized hydrocarbonoclastic bacteria prevailing in seawater around a port in the Strait of Malacca. **PLoS One.** v. 8, n. 6, p. e66594, 2013. doi: 10.1371/journal.pone.0066594.

UKALSKA-JARUGA, A.; DABAENE, G.; SMRECZAK, B. Dissipation and sorption processes of polycyclic aromatic hydrocarbons (PAHs) to organic matter in soils amended by exogenous rich-carbon material. **J Soils Sediments.** v. 20, p. 836–849, 2020. doi: 10.1007/s11368-019-02455-8.

URVOY, M.; LABRY, C.; L'HELGUEN, S.; LAMI, R. Quorum sensing regulates bacterial processes that play a major role in marine biogeochemical cycles. **Front Marine Sci.** v. 9, p. 834337, 2022. doi: 10.3389/fmars.2022.834337.

VALDERRAMA, C.; ALESSANDRI, R.; AUNOLA, T.; CORTINA, J.L.; GAMISANS, X.; TUHKANEN, T. Oxidation by Fenton's reagent combined with biological treatment applied to a creosote-contaminated soil. **J Hazard Mater.** v. 166, n. 2-3, p. 594-602, 2009.

VANDRISSE, C.M.; LIPSH-SOKOLIK, R.; KHERSONSKY, O.; NEWMAN, D.K. Computationally designed pyocyanin demethylase acts synergically with tobramycin to kill recalcitrant *Pseudomonas aeruginosa* biofilms. **PNAS.** v. 118, n. 12, p. e2022012118, 2021. doi: 10.1073/pnas.2022012118.

VARJANI, S.J. Microbial degradation of petroleum hydrocarbons. **Bioresour Technol.** v. 223, p. 277-286, 2017.

VARJANI, S.; UPASANI, V.N.; PANDEY, A. Bioremediation of oily sludge polluted soil employing a novel strain of *Pseudomonas aeruginosa* and phytotoxicity of petroleum hydrocarbons for seed germination. **Sci Total Environ.** v. 737, p. 139766, 2020. doi: 10.1016/j.scitotenv.2020.139766.

VIANA, A.A.G.; MARTINS, R.X.; FERREIRA, G.F.; ZENAIDE-NETO, H.; AMARAL, I.P.G.; VASCONCELOS, U. *Pseudomonas aeruginosa* and pyocyanin negatively act on the establishment of Enterobacteriaceae biofilm on a ceramic surface. **Int J Eng Res Appl.** v. 7, n. 8, p. 23-30, 2017.

VIANA, A.A.G.; OLIVEIRA, B.T.M.; CAVALCANTI, T.G.; SOUSA, K.A.; MENDONÇA, E.A.M.; AMARAL, I.P.G.; VASCONCELOS, U. Correlation between pyocyanin production and hydrocarbonoclastic activity in nine strains of *Pseudomonas aeruginosa*. **Int J Adv Eng Res Sci.** v. 5, n. 7, p. 212-223, 2018.

WANG, C.; WANG, Y.; HERATH, H.M.S.K. Polycyclic aromatic hydrocarbons (PAHs) in biochar–Their formation, occurrence and analysis: A review. **Org Geochem.** v. 114, p. 1-11, 2017.

WANG, K.; KAI, L.; ZHANG, K.; HAO, M.; YU, Y.; XU, X.; YU, Z.; CHEN, L.; CHI, X.; GE, Y. Overexpression of phzM contributes to much more production of pyocyanin converted from phenazine-1-carboxylic acid in the absence of RpoS in *Pseudomonas aeruginosa*. **Arch Microbiol.** v. 202, n. 6, p. 1507-1515, 2020. doi: 10.1007/s00203-020-01837-8.

WEISER, R.; GREEN, A.E.; BULL, M.J.; CUNNINGHAM-OAKES, E.; JOLLEY, K.A.; MAIDEN, M.C.J.; HALL, A.J.; WINSTANLEY, C.; WEIGHTMAN, A.J.; DONOGHUE, D.; AMEZQUITA, A.; CONNOR, T.R.; MAHENTHIRALINGAM, E. Not all *Pseudomonas aeruginosa* are equal: strains from industrial sources possess uniquely large multireplicon genomes. **Microb Genom.** v. 5, n. 7, p. e000276, 2019. doi: 10.1099/mgen.0.000276.

WHOOLEY, M.A.; McLOUGHLIN, A.J. The regulation of pyocyanin production in *Pseudomonas aeruginosa*. **Eur J Appl Microbiol Biotechnol.** v. 15, p. 161-166, 1982.

XUE, S-W. ; TIAN, Y-X. ; PAN, J-C. ; LIU, Y-N. ; MA, Y-L. Binding interaction of a ring-hydroxylating dioxygenase with fluoranthene in *Pseudomonas aeruginosa* DN1. **Sci Rep.** v. 11, p. 21317, 2021. doi: 10.1038/s41598-021-00783-9.

YAKOVLEVA, E.V.; GABOV, D.N.; BEZNOSIKOV, V.A.; KONDRATENOK, B.M.; DUBROVSKIY, Y.A. Accumulation of PAHs in tundra plants and soils under the influence of coal mining. **Polycycl Aromat Compd.** v. 37, n. 2-3, p. 203-218, 2016. doi: 10.1080/10406638.2016.124408.

YAN, S.; WU, G. Can biofilm be reversed through quorum sensing in *Pseudomonas aeruginosa*? **Front Microbiol.** v. 10, p. 01582, 2019. doi: 10.3389/fmicb.2019.01582.

YAN, S.; WU, G. Reorganization of gene network for degradation of polycyclic aromatic hydrocarbons (PAHs) in *Pseudomonas aeruginosa* PAO1 under several conditions. **J Appl Genet.** v. 58, n. 4, p. 545-563, 2017. doi: 10.1007/s13353-017-0402-9.

ZHANG, L.; CHIANG, W.C.; GAO, Q.; GIVSKOV, M.; TOLKER-NIELSEN, T.; YANG, L.; ZHANG, G. The catabolite repression control protein Crc plays a role in the development of antimicrobial-tolerant subpopulations in *Pseudomonas aeruginosa* biofilms. **Microbiology.** v. 158, p. 3014–3019, 2012. doi: 10.1099/mic.0.061192-0.

ZHANG, X-X.; CHENG, S-P.; ZHU, C-J.; SUN, S-L. Microbial PAH-degradation in soil: Degradation pathways and contributing factors. **Pedosphere.** v. 16, n. 5, p. 555-565, 2006.

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