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 to 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 synthetize 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.

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 (KCHARAKA 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 20th 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
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

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


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 (JOHNSON et 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).
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 µg/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-RHDα genes encode dioxygenases (MUKHERJEE et al., 2017). In particular, the PAH-RHDα 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 PO$_4^{3-}$ and 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 ($\text{H}_2\text{O}_2$) and the hydroxyl radical (OH•) (SINHA et al., 2015; BRITIGAN et al., 1992). There is an intrinsic dependence on the availability of $\text{H}_2\text{O}_2$ to produce OH• (BABUPONNUSAMI; MUTHUKUMAR, 2014). In addition, the hydroxyl radical has a high oxidizing potential ($E_\text{y} = 2.8V$), which enables it to attack many organic compounds (KAHOUSSH 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 H₂O₂, 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).

\[
H_2O_2 + Me \rightarrow OH^- + OH^- + Me_{(oxid)} \quad (Eq. 1)
\]

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), \textit{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 H₂OF₂, 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 \textit{P. aeruginosa}?

Pyocyanin is beneficial for \textit{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 ΔphzM 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 \textit{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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