Conjecturas

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Hydrocarbonoclastic activity in bacterial biofilms: A systematic study emphasizing pseudomonads

Atividade hidrocarbonoclástica em biofilmes: um estudo sistemático enfatizando pseudomonadas

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ABSTRACT

Pseudomonas aeruginosa is a ubiquitous fluorescent, rod-shaped pseudomonad, with a high metabolic capacity, and potential for application in processes to remove recalcitrant compounds such as petroleum hydrocarbons (PHC) from the environment. The bacterium persists in sites with highly selective pressures such as those contaminated by PHC. One of the bacterium's strategies is to colonize biofilms which enhance its protection from toxic compounds and favor oil uptake. It is the most prevalent microbe at sites impacted by PHC owing to the use of aliphatic hydrocarbons to form biofilms and other metabolites crucial for the uptake and degradation of crude oil. *P. aeruginosa* could be useful in biofilm-mediated bioremediation; however, it has been poorly explored in the last ten years. This systematic study addresses recent research on the application of *P. aeruginosa*/pseudomonads biofilms in bioremediation The studies come from Asia and Africa and emphasized the formation of biofilm by *P. aeruginosa* and other pseudomonads as crucial elements in the detoxification process of the environment.

Keywords: Biofilm-mediated bioremediation; Oil hydrocarbons; *Pseudomonas aeruginosa*.

RESUMO

Pseudomonas aeruginosa é uma pseudomonada fluorescente ubíqua, em forma de bastonete, com alta capacidade metabólica e potencial para aplicação em processos de remoção de compostos recalcitrantes do ambiente, tais como hidrocarbonetos de petróleo (HCP). A bactéria persiste em locais com pressões altamente seletivas, como os contaminados por estes compostos. Uma das estratégias da bactéria é colonizar biofilmes, pelos quais a proteção contra compostos tóxicos é aumentada, favorecendo a absorção do óleo. Ela é a bactéria mais prevalente em locais impactados pelo HCP porque utiliza hidrocarbonetos alifáticos para formar biofilmes, bem como outros metabólitos cruciais para a absorção e degradação do petróleo bruto. Assim, *P. aeruginosa* pode ser útil na biorremediação mediada por biofilme; entretanto, tem sido pouco explorada nos últimos dez anos. Este estudo sistemático aborda pesquisas recentes sobre a aplicação de biofilmes de *P. aeruginosa*/pseudomonadas na biorremediação. Os estudos foram asiáticos e africanos e enfatizaram a formação de biofilme por *P. aeruginosa* e outras pseudomonadas como elementos cruciais no processo de desintoxicação do ambiente.

Palavras-chave: Biorremediação mediada por biofilme; Hidrocarbonetos do petróleo, *Pseudomonas aeruginosa*.

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INTRODUCTION

The use of oil has made this compound a strategic and notable feedstock representing one of the most important commodities in the global economy (SAYED et al., 2021). Despite the care taken to comply with safety standards required at each stage, from extraction to storage, as well as the implementation of emergency protocols and strategies to try to minimize oil spills, petroleum hydrocarbons are among the main and most common environmental pollutants (ŁAWNICZAK et al., 2020; FUENTES et al., 2014). Crude oil processing activities in the hydrocarbon industry release hazardous organic compounds whose toxicity and chemical characteristics can cause long-term ecological impacts (BARROM et al., 2020; VARJANI et al., 2017).

The hydrocarbons found in oil are chemically stable molecules, which increases their persistence and recalcitrance (LADINO-ORJUELA et al., 2016). These molecules, however, can be assimilated by microorganisms (NIKOLOVA and GUTIERRES, 2020); at least 175 bacterial genera have been described as hydrocarbonoclastic, i.e., microbes that use oil hydrocarbons as the sole or preferred source of carbon and energy (McGENITY et al., 2012). On the other hand, under natural conditions, the presence of hydrocarbonoclastic microbes is very low compared to other microbial groups. They become dominant as the degree of contamination by oil hydrocarbons increases (DOURADO et al., 2018).

Hydrocarbon mineralization is a process that occurs preferentially under aerobic conditions. The isolation of aerobic bacteria sheds light on the management of detoxification of oil-impacted sites (DASHITI et al., 2015). In addition, there is a correlation between several oil-utilizing bacteria and diazotrophs, indicating that diazotrophic bacteria may be important in oil attenuation, both in nitrogen-rich and nitrogen-free environments (RADWAN et al., 2010). Additionally, the growth of nitrogen-fixing microbes is reflected in the increase of the biodegradation of nitrogen-free hydrocarbons, suggesting that nitrogen fixation serves as a mechanism that contributes to the detoxification of oil-contaminated sites (SHIN et al., 2019).

In terms of hydrocarbonoclastic/diazotrophic microbial diversity, some prokaryotes prevail, among them, pseudomonads, a large number of free-living bacteria of the family Pseudomonadaceae that live primarily in soil, fresh water, seawater and plants, of which *Pseudomonas* spp. are the most important microorganisms (KERSTERS et al., 1996). Because of various physiological and nutritional factors, *P. aeruginosa* is

the representative species of pseudomonads (WU et al., 2018). It is a motile gram-negative and ubiquitous rod-shaped aerobic bacterium. Because *P. aeruginosa* produces different phenazines, it is member of the group of fluorescent pseudomonads. Furthermore, the bacterium has a remarkable metabolism that helps it to adapt to the different environmental conditions to which the species is exposed (GONÇALVES and VASCONCELOS, 2021; MAVRODI et al., 2006). The metabolic capacity of *P. aeruginosa* contributes to its persistence under stressful conditions in different environments (MORADALI et al., 2017).

Because *P. aeruginosa* can use a variety of carbon sources, it is commonly found in oil-contaminated sites (LIMONGI et al., 2020; VIANA et al., 2018). One of the metabolic strategies performed by *P. aeruginosa* is the ability to form biofilms, i.e., a three-dimensional structure adhered to a surface through a polymer matrix (THI et al., 2020). Biofilm formation favors a rapid and efficient reduction of oil hydrocarbons; cell imprisonment is advantageous because it attributes greater tolerance to the contaminant, compared to free-floating planktonic cells (MITRA and MUKHOPADHYAY, 2016), and increases the chance of adaptation and survival under toxic conditions (SINGH et al., 2006).

In addition, the production of signaling molecules (autoinducers) by cells in the biofilm facilitates the expression of enzymes and biosurfactants that regulate the degradation of petroleum hydrocarbons (LAHIRI et al., 2021). Given this, biofilm-mediated bioremediation provides advantages in terms of cost-effectiveness, formation of assimilable intermediate metabolites and the possibility of complete transformation of the pollutant (HASSANSHABIAN et al., 2020; CHEN et al., 2017). Based on this, this study aimed to identify studies, published in the ten years before 2021, in which *P. aeruginosa* played a leading role in the biodegradation of petroleum hydrocarbons, emphasizing the role of biofilm formation in the process

MATERIALS AND METHODS

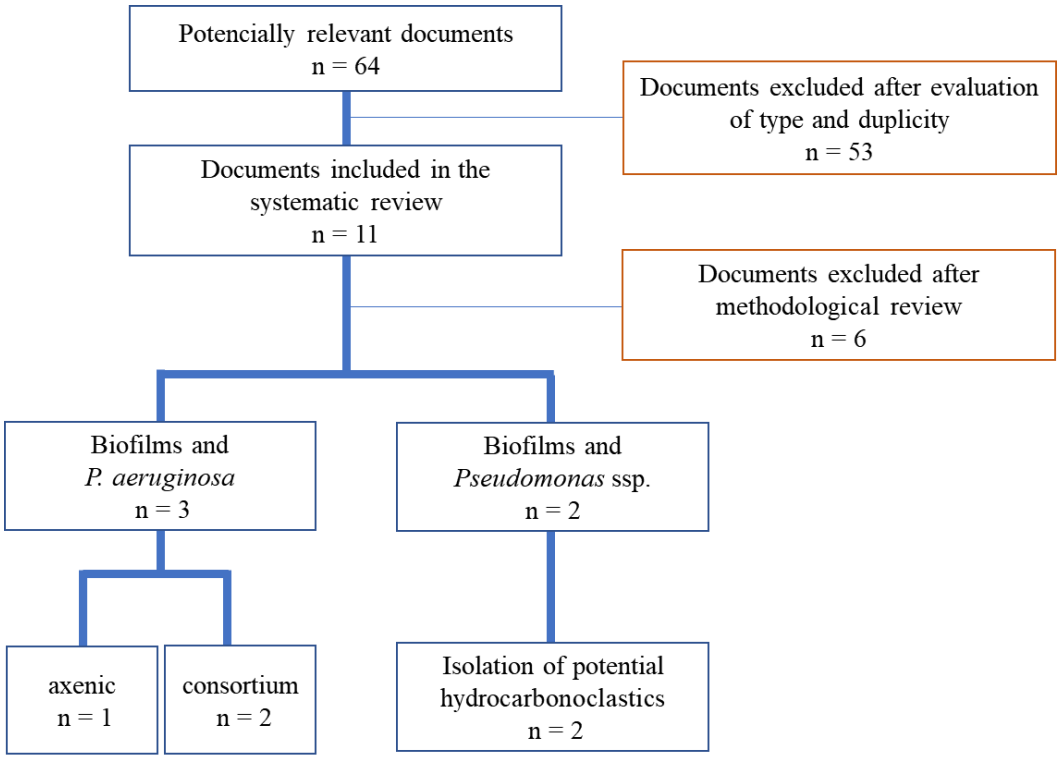
The search and selection of articles was carried out in October 2021, using the criteria established by the methodological guidelines for the elaboration of systematic reviews and meta-analysis of the Ministry of Health of Brazil (2012). Three databases were used, among the most accessed in Brazil, the Brazilian platform Periódicos CAPES and the American platforms National Center for Biotechnology Information (NIH) and

Google Scholar. These were selected because of their large collection of articles and intuitive filtering tools. The keywords searched were “biofilm”, “hydrocarbonoclastic”, “heavy oil” and “*Pseudomonas aeruginosa*”, anywhere in the text of original articles, published in English between October 15, 2011 and October 15, 2021. English was chosen in order to identify articles within the reach of readers worldwide. In this study, only documents with access to the full text were considered, excluding review articles of any type, as well as technical notes, letters, editorials, theses, abstracts, articles in press, pre-prints and material published in books.

RESULTS

Figure 1 summarizes the evolution of the process of including documents in this research. In the initial screening, a total of 64 articles were gathered, 24 of which were obtained from the Periódicos CAPES, 20 documents from the NIH and 20 from the Google Scholar.

Figure 1 – Diagram of the document selection process



After applying the exclusion criteria, identifying duplicates and articles dealing with bioremediation, 11 documents remained, of which only five addressed investigations

involving pseudomonad biofilms in the process. *Pseudomonas aeruginosa* was cited in only three of them, as an axenic culture (PATHAK et al., 2017) or mixed with consortia (LI et al., 2017; Al-MAILEM et al., 2014). The other two works identified *Pseudomonas* ssp. (as pseudomonads) in studies on the use of natural diversity in hydrocarbon-contaminated sites (AMER et al., 2015; CHIKERE et al., 2012).

The studies covered three areas of knowledge: Environmental Sciences, Environmental Biotechnology and Biological Sciences, reporting their results in two different contexts (Table 1). The first scenario constituted a baseline investigation (AMER et al., 2015); The second, an applied investigation, by prospecting the hydrocarbonoclastic potential of *P. aeruginosa*/pseudomonads (LI et al., 2017; PATHAK et al., 2017; AL-MAILEM et al., 2014; CHIKERE et al., 2012).

Table 1 – Scope and remarks of studies on the biodegradation of hydrocarbons mediated by *Pseudomonas* spp. biofilm (2012-2017)

Scope	Remark	Reference
Biofilms of hydrocarbonoclastic isolates of *Pseudomonas aeruginosa*		
Evaluation of biofilm-forming hydrocarbonoclastic microbes recovered from sewage effluents and cultured on slides with different surface areas, aiming at potential use for the removal of contaminants.	Biofilms developed on the surface of slides and removed n-hexadecane, phenanthrene and crude oil. The proportion of hydrocarbons removed was related to the size of the substrate surface area. The addition of thioglycolate inhibited the microbiota and consequently the hydrocarbon biodegradation rate. The *Pseudomonas* genus led the more than ten other hydrocarbonoclastic bacterial genera detected in the biofilms. *P. aeruginosa* was the second most dominant species in all tested conditions. These results indicate the potential of biofilms to mediate sewage bioremediation.	Al-Mailem et al. (2014)
Comparison of three adhesion substrates installed in a columnar reactor to evaluate the removal of gasoline additive methyl tert-butyl	Under continuous flow, the MTBE were adsorbed through the column and became more bioavailable. Biofilms were formed on the three substrates, however the most stable biofilm occurred with the PPy/GAC	Li et al. (2017)

ether (MTBE), polypyrrole (PPy) composite 0.2 M, added to granular activated carbon (GAC); and PPy and GAC separately.	combination, which was subjected to treatments over 30 to 60 days with long-term MTBE concentrations ranging from 0.1 to 50 mg/L. At the end of the process, values lower than the USEPA recommended discharge limit (< 20 µg/L) were reached. *Pseudomonas aeruginosa* and *Acinetobacter pittii* were the dominant bacteria, and also identified as responsible for the degradation of MTBE.	
Generation of a biofilm (bioflocculant) synthesized from n-hexadecane by the strain *Pseudomonas aeruginosa* IASST201 as a substitute for chemical compounds used in the removal of heavy metals. As a secondary objective, verification of whether the production of bioflocculants by the strain was dependent on the complex mixture of 34 hydrocarbons present in the crude oil or on a particular hydrocarbon.	n-hexadecane was identified as the most effective hydrocarbon in the production of exopolysaccharide from the biofilm, characterized by 3-sugar monomers (D-xylose, D-rhamnose, D-sucrose), D-glucuronic acid and traces of N-acetyl D-glucosamine. Additionally, more than 83% of n-hexadecane was degraded when a higher concentration of biofilm was produced, often surpassing crude oil degradation. These indicate that *P. aeruginosa* IASST201 had the highest polymer matrix production activity in the presence of a single aliphatic hydrocarbon rather than a complex carbon source. The increase in hydrophilic compounds during the use of n-hexadecane was efficient in the removal of metals: $Ni^{2+} > Zn^{2+} > Cd^{2+} > Cu^{2+} > Pb^{2+}$.	Pathak et al. (2017)
Biofilms of hydrocarbonoclastic isolates of *Pseudomonas* spp.		
Use of seven conditions whose bioreactors contained 1 L of seawater added to crude oil and 0.2 g of anthracene, inoculated with 1 kg of marine sediment to investigate the hydrocarbonoclastic potential from natural marine microbial	After 56 days, the concentrations of total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) in the reactors were reduced by 94-95% and 93-98%, respectively, in the presence of inorganic nitrogen and phosphorus salts. The TPH content was < 14.9 mg/L while PAHs < 6.8 mg/L. Fifty-six hydrocarbonoclastic	Chikere et al. (2012)

communities, stimulated by the addition of different concentrations of nitrogen and phosphorus.	bacterial isolates were observed, among which *Pseudomonas* spp., in equal proportions as *Alcanivorax* spp. and *Arthobacter* spp., were the most dominant microbes (13.46% each one). The authors concluded that natural microbial communities present in marine impacted sediments have the innate ability to detoxify sites where hydrocarbons are present.	
Investigation of bacterial communities installed in marine sediments from different stations located in El-Max Bay (Egypt) to unravel the pattern of bacterial diversity, ecological interactions and the hydrocarbonoclastic potential to exploit these microbes in the bioremediation of marine highly contaminated sediments.	The association of molecular and statistical tools from collected sediment samples indicated a significant correlation between biotic and abiotic factors in hydrocarbon-polluted ecosystems as driving forces for modulating biofilm formation and bacterial microbiome structure in marine sediments. Some of these were: texture soil, total organic content, and concentration of metals, particularly Cu, Fe and Zn. More than 10 bacterial genera have been identified with *Pseudomonas* spp. as the most prevalent microbes (relative abundance in samples >88%). Two *Pseudomonas* species were detected, *P. stutzeri* and *P. knackmussii*, whose relative abundances were, respectively, 8 and 4%. In addition, all isolates were detected as able to produce strong or weakly adherent biofilms. The greater hydrophobicity of the cells favored the expression of important factors in the degradation of the oil, such as the synthesis of biosurfactants. Biofilms were more present in the samples with the highest levels of hydrocarbons.	Amer et al. (2015)

It is noteworthy that, also considered in these same studies were other hydrocarbonoclastic bacteria of numerous genera reported as biofilm producers, whose application of bacterial biofilms in bioremediation was reported to bring good results. The present study was reserved for pseudomonads only, with special attention to *P. aeruginosa*, assuming its importance as a degrading agent for petroleum hydrocarbons.

The studies were carried out in institutions on two continents; Asia was the region with the highest concentration of research, 60% (China, Kuwait and India). The remaining studies were conducted in Africa (Nigeria and Egypt). No studies were identified from Europe, Oceania nor the Americas. Most articles were published between 2012 and 2015, while 2017 was the year with the highest number of publications. According to the methodology used, the search did not find material published in the early 2020s; this, however, does not represent a lack of interest on the topic, because there is a growing increase in studies referring to the biofilm-mediated bioremediation using natural bacterial consortia and or mixed communities, as observed among the 59 excluded documents.

DISCUSSION

The aim of this study was to explore the available material published in the last ten years on the three largest search platforms used by Brazilian students and researchers, on the potential use of *P. aeruginosa* biofilms mediating bioremediation. This bacterium is one of the most prevalent species in sites contaminated by oil hydrocarbons (AMER et al., 2015; AL-MAILEM et al., 2014). It is known to be versatile from a metabolic point of view (FRIMMERSDORF et al., 2004). In addition to this, the current model of biofilm dynamics is based on the behavior of the species (PASSOS DA SILVA et al., 2017).

The authors' intention was to exemplify *P. aeruginosa* as one of the most important bioremediation agents, however, this was not observed in the documents found in this search. An exception was found in two reports, coincidentally published in the same year (PATHAK et al., 2017; LI et al., 2017). These materials also represent the most recent works on the subject, up to date of the ending of this study. Only Pathak et al. (2017) failed to discuss the detoxification of sediments contaminated by oil hydrocarbons resulting from a metabolic cooperation between an established microbial diversity in biofilms. Furthermore, the researchers observed that the *P. aeruginosa* strain preferentially chose a single hydrocarbon as a nutrient for the synthesis of the monomer

components of the exopolysaccharide. This substrate preference occurs at the expense of the complex mixture of long-chain aliphatic hydrocarbons or polycyclic aromatic hydrocarbons, which require more energy expenditure for the conversion of these substrates into assimilable compounds.

Because the literature characterizes *P. aeruginosa* as an opportunistic pathogenic microbe (PALLERONI, 2010), our opinion is that the bacterium has not been well studied for this reason. No study has focused on it as an opportunistic microbe. In all documents looked at as part of our internet research, *P. aeruginosa* was included as part of a diversity, without addressing its particular role as an obstacle in the process of removing toxic contaminants, as has been widely reported in the literature (BAIG et al., 2021; CAVALCANTI et al., 2019). Additionally, it should be noted that *P. aeruginosa* is recognized as a keystone species in the degradation processes of numerous chemical compounds in both soil and water (CENTLER et al., 2020). Regarding the *P. aeruginosa* pathogenicity, a comprehensive study carried out in the USA showed that 330 isolates of autochthonous pseudomonads were able to inhibit clinical strains of *P. aeruginosa* that occurred in natural environments, such as soil and fresh water. This means that clinical strains are susceptible to other pseudomonads, including wild-type *P. aeruginosa*, although specific mechanisms have not been discussed by the researchers (CHATERJEE et al., 2017).

By proposing that natural hydrocarbonoclastic bacterial communities exhibit detoxification potentials in oil-impacted environments, biofilm has begun to be seen as a bioremediation strategy (PATHAK et al., 2017). A number of benefits have been attributed to microbes living in biofilms in terms of their actions in transforming organic pollutants, such as tolerance to environmental stresses as well as toxic chemicals (BOLES et al., 2004), cellular communication (SARKAR and CHAKRABORTY, 2008), exchange of genetic material (WOLCOTT et al., 2013), diversity in electron acceptors (CHEN et al., 2013), variation in growth rate by induction of different metabolite states in cells within the biofilm (COSTERTON, 1999), transport of nutrients and waste products through channels (WANG and OYAIZU, 2011), production of surfactants (RODRIGUEZ and BISHOP, 2008) and gradient formation and redox potential (VERHAGEN et al., 2011).

Biofilm-forming hydrocarbonoclastic bacteria have developed mechanisms to improve the ability of cells to adhere to surfaces, altering surface components and

secreting surfactants, thereby increasing access to hydrocarbons (XU et al., 2018). Furthermore, the hydrophobicity of the cells guides the type of adhesion of the bacteria to the substrate. In *P. aeruginosa*, weak adhesion is common (DELIGIANNI et al., 2010). This characteristic is attributed to the evolution and adaptation of the species as it facilitates detachment to initiate new colonization and hydrocarbon uptake, avoiding competition with other hydrocarbonoclastic bacteria (AMER et al., 2015). Thus, the inherent durability of *P. aeruginosa* biofilms ensures greater cycling of nutrients in certain microsystems (EDWARDS and KJELLERUP, 2013), as well as the potential for their exploitation in bioremediation. However, the *in situ* application of *P. aeruginosa* still needs to be better explored

CONCLUSION

There is little information on the potential application of *P. aeruginosa*/pseudomonads biofilm-mediated bioremediation, although the potential of the bacterium has not been entirely neglected. The studies selected by the criteria adopted in this study emphasized and discussed the process of detoxification of oil hydrocarbons in effluents or sediments under a multispecies view, i.e., natural microbial communities, highly diverse and abundant. Their coexistence may guarantee a myriad of metabolic strategies that are waiting to be explored in the search to optimize the cost reduction of bioremediation. Our conclusion is that *P. aeruginosa* should attract more interest by assuming the role of a keystone species in terms of transforming hydrocarbons in the environment.

CONFLICTS OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

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