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MICROBIAL DEGRADATION FOR THE REMEDICATION OF SOILS CONTAMINATED BY CRUDE OIL IN PERU: A SYSTEMATIC REVIEW OF THE LITERATURE

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Microbial degradation for the remediation of soils contaminated by crude oil in Peru: A systematic review of the literature

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Abstract. In this research, a systematic literature review is carried out whose objective is to identify, compare and analyze the main fundamentals to be considered in order to face the problematic of remediation of soils contaminated by crude oil in order to establish and achieve a high efficiency of this. This work was elaborated through a comprehensive analysis of the various suitable scientific articles that provide a more comprehensive basis in relation to what is discussed in this research, taking as a basis the results that were optimal in the remediation of soils in terms of their various experiments made that were successful in the realization. Therefore, finally, it is obtained that the various technologies used for the remediation of the contaminated soil should not be treated only at the laboratory level but also at the field level due to the fact that there is a great gap in the treatment of the soil due to the conditions and variables that change significantly, thus reducing their remedial application in contaminated soil, presenting a minimal environmental impact and high investment costs in large-scale projects.

1 Introduction

In Peru, oil activity in hydrocarbon extraction began in 1971 with the discovery of the first oil wells in the Peruvian Amazon, motivating the construction of the North Peruvian oil pipeline, whose extension of its infrastructure reaches approximately 1,106 km of pipes that crosses the Amazon and Peruvian Andes to carry crude oil to the limit with the Pacific Ocean [1].

Crude oil, considered the “black gold” in the Peruvian Amazon, represents 85% of the energy consumption in the country, added together with those from natural gas and coal; given its importance in the country, Perupetro made a statistical estimate related to the oil consumed, which reaches around 1,577 million barrels since 1994 being a very important source over the years due to the constant energy demand it has. However, although it estimates a production of 1,577 million barrels, oil production is actually 763 million barrels; this amount being only a national production of 48%, making Peru a country without energy autonomy.

Given the magnitude of the problem, a subordinate indicator of an idealistic image regarding the reality of oil was evidenced, generating a controversial context about extractive activities, the legal framework and the stakeholders involved [1].

This is mainly due to the fact that in this part of Peru, is considered an area of oil exploitation this is due to the often emergency situations of oil spills or incidents of the companies in charge of extractive activities. One of the sources of contamination are hydrocarbons and oils derived from petroleum that generate harmful damage mainly to soils, waters, the structure of ecosystems and bioprocesses [2]. Evidencing accidental signs whose

concern about environmental sustainability is latent since, consequently, they cause damage to microorganisms, plants, animals and humans. An example of these is the contamination of water and soil through the underground injection of oil wells for oil extraction, the soil affected by the splash caused by extraction, accidental spills in water and soil, the smoke generated by the refineries, the water used in the oil and petroleum processes that are discarded from there without an adequate form of treatment.

This study is based on the collection of data from the oil lots in the north-east of Peru, specifically, the Peruvian jungle; where as a result of their activities in the pipeline systems, tank cleaning, oil-water separators and transfers of crude oil to storage tanks, large amounts of pollutants are generated [3]. This type of oil spill has an imminent and profound effect on the environment that can seep into the soil to the water table and groundwater, contaminating it to the point of making it irremediable for its recovery [3]. We can review this in Fig 1 where a segmented graph is shown with the main causes generated by the contamination of Amazonian soil by crude oil, according to the National Human Rights Coordinator (CNDDHH).

According to another study carried out by the same entity, between 2000 and 2019 around 474 oil spills were identified in the Peruvian Amazon. 65.4% of said spills were due to corrosion of the pipelines, operational failures or unsafe conditions of the facilities, 28.8% caused by human actions, being among the main actions various attacks or sabotage caused by people outside the facilities, and 5.8% due to natural causes [1], as show in Fig 2.

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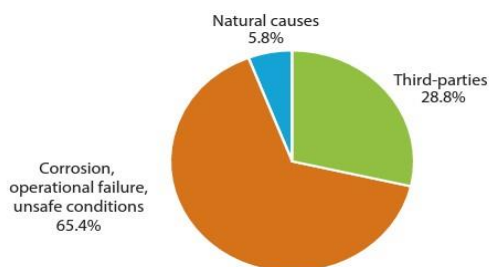


Fig. 1. Percentage of spills by cause in Amazon batches and the ONP (2000 -2019). Font: OEFA y OSINERGMIN.

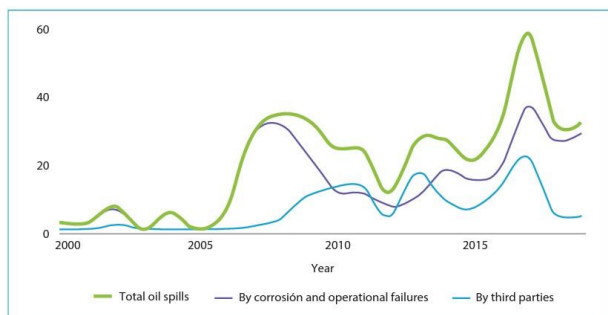


Fig. 2. Total number of spills in the Amazon lots and the ONP (2000-2019). Font: OEFA y OSINERGMIN.

Therefore, the initiative taken for this research work is aimed at the use of a bioremediation process of contaminated soils, opting for microbial remediation as a more efficient process, which consists of the use of bacterial strains under the study of its functional structure. It was shown that its microbial enzymatic activity is responsible for the biodegradation of contaminants, being useful for the development of this research [4]. This allows, through the bioremediation cycle, the bacterial strains to reduce the concentration of hydrocarbons and petroleum-derived oils; being an agent treating the recovery and regeneration of contaminated soil, favoring the restoration of the ecosystem and the biota of the site where the greatest environmental concern can be registered [5]. Therefore, we will emphasize addressing this issue in our research; for which we present the following main problem: What type of research on microbial degradation for the remediation of soils contaminated by crude oil, have been carried out in recent years in America, Asia and Africa.

For this we must deepen the research on the chosen bioremediation methods; Although all types of bioremediation carry out the elimination of the toxins present in the soil using living beings, there are differences between them, which consist of the use of the hydrocarbon degrading agent; within these are Bioaugmentation and Biostimulation. Bioaugmentation is responsible for introducing exogenous microorganisms that are capable of degrading hydrocarbons in the soil [6].

Biostimulation in which nutrients are added to the affected soil in order to stimulate the growth of microorganisms and thus increase their population. These two methods will be reviewed and evaluated according to their degree of impact for our research.

In effect, due to the uncertainty of the achievement of this type of bioremediation compared to the rest of the types, it generates that the general objective is to analyze the effectiveness obtained from the methods used in investigations that deal with the remediation of soils contaminated by oil. oil in America, Asia and Africa in recent years, through a systematic review of the literature.

In this research, the main fundamentals that should be considered for the development of the experimentation of the different types of bioremediation were identified, compared and analyzed; as well as the reports for months of the experimentation of the contaminated soil for its bioremediation. The articles reviewed on the experiences shown in bioremediation, the phases of bioremediation, and their effectiveness of use in each type of bioremediation, were identified through a systematic review of the literature. The results of this research were obtained based on the analysis of the various scientific articles and technical documents published between 2015 and 2021.

Given the diversity of the articles reviewed, in this research a general concept of treatment of contaminated soil is not used to limit the search, but the concepts of treatment of contaminated soil are accepted according to each author who has dealt with the topic addressed in the articles consulted.

This scientific article was developed based on the methodology of systematic review of the literature that was carried out with the following contributions: (1) analyze the laboratory studies of contaminated soils and how they define the development of the type of bioremediation used, (2) analyze the main stages of the experimentation of the various types of bioremediation used (3) and contrast the optimal type of bioremediation according to its level of remediation on the contaminated soil.

2 Methodology

2.1. Type of study

This research was carried out using a qualitative approach, this is because the research aims to present results of the systematic review of the literature about soil contamination by crude oil; with the purpose of having an impact on this research topic and its subtopics that are the subject of study by the scientific community. The type of research is non-experimental, despite the fact that a scientific assessment was made for a literary purpose, information gathering and the evaluation of scientific articles according to the impact or significance that it has.

Finally, this research is of longitudinal design where relevant information was collected from the scientific articles in English and Spanish consulted on the remediation of soils contaminated by crude oil since 2015 and its development to the present, including the various research subtopics.

2.2 Process

The methodology for the systematic review of scientific literature is developed based on the information collected from articles, scientific journals, etc., indexed in bibliographic repositories with scientific contribution, related to science, technology and medicine such as Scielo, Springer, Hindawi, Redalyc, ScienceDirect, Scopus and Web of Science (WOS). Likewise, this research begins with the definition of the topic, subject of the present; then, the selection criteria of the scientific articles are developed and the scientific systematic review is carried out; the same that is displayed in the search, selection, processing and technical review. Finally, the conclusions reached in the systematic review carried out are provided.

2.2.1. Classification of scientific articles to be included in the study

The classification method used is according to the following criteria that we will show below.

- Being in an indexed academic journal.
- Written in English or Spanish.
- Being in the quartiles Q1, Q2 or Q3.
- Contain less than thirty-five pages
- Proven results with a specific methodology.
- Have been published between 2015 and the present.
- Deal with the remediation of soils contaminated by crude oil in general and the microbial degradation of contaminated soils.
- Have experimental studies at the laboratory and field level.
- Be referred to microbial degradation in soils affected by crude oil.

Then, in the article we proceeded to use the bibliographic reference management tool called Mendeley, a tool in which the abstracts of each article could be read to see if they meet or not the requirements specified as search criteria and selection of scientific articles to be collected for systematic review of the literature. Finally, after reviewing the scientific articles, only twenty remained, which were subsequently evaluated to see the quality of their content and their compliance with the main objective of ensuring the reliability and integrity of the information shown in the review. systematics of the final literature. For this, criteria were used that allowed reaching a quality measurement of each scientific article; assigning them a classification and possible qualification score of "Excellent" = 2, "Fair" = 1 and "Poor" = 0.

That is why only those investigations whose quality score was higher than 12 (75% of the perfect score) could be accepted to subsequently collect and synthesize the data extracted from said selected scientific articles. Then, the following will show Fig 3 with the classified articles

and Fig 4 with the details of these articles, previously classified.

Qualifying criteria	SA1	SA2	SA3	SA4	SA5	SA6	SA7	SA8	SA9	SA10	SA11	SA12	SA13	SA14	SA15	SA16	SA17
The summary presents the research, the problems, objectives and the methodology developed, as well as the most relevant findings and conclusions.	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
It describes the problems that give rise to the research and bases the need to solve them.	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Clearly defines the purpose of the research.	2.00	2.00	2.00	2.00	1.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
The introduction describes the knowledge framework and is based on relevant bibliographic references. Identifies the variables and dimensions of the research.	1.00	2.00	2.00	2.00	2.00	2.00	2.00	1.00	2.00	2.00	2.00	2.00	1.00	1.00	1.00	2.00	2.00
The methodology is sufficiently described, detailing the methods and techniques used.	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	1.00	2.00
The results are fully exposed.	2.00	2.00	2.00	2.00	2.00	2.00	2.00	1.00	2.00	1.00	2.00	2.00	2.00	2.00	2.00	2.00	1.00
The discussion explains the behavior of the results in light of their trends and their comparison with the results of other similar research.	1.00	2.00	1.00	2.00	1.00	1.00	2.00	2.00	1.00	2.00	2.00	2.00	1.00	2.00	1.00	2.00	2.00
TOTAL	12.00	14.00	13.00	14.00	12.00	13.00	14.00	12.00	13.00	13.00	14.00	14.00	12.00	13.00	12.00	13.00	13.00

Fig 3. Qualification of the selected scientific articles.

Nº SA	Title	Author(s)
SA1	Efectos ambientales de los hidrocarburos. Una revisión.	Ortiz Díaz, D. (2019)
SA2	Microbial degradation of total petroleum hydrocarbon in crude oil polluted soil ameliorated with agro-wastes	Agbor, R. B., Antai, S. P., & Nkanang, A. J. (2018).
SA3	Bioremediation of hydrocarbon degradation in a petroleum-contaminated soil and microbial population and activity determination	Wu, M., Li, W., Dick, W. A., Ye, X., Chen, K., Kost, D., & Chen, L. (2017).
SA4	Bioremediation of Contaminated Soil. Introduction to Petroleum Biotechnology	Speight, J. G., & El-Gendy, N. S. (2018).
SA5	Biodegradation of diesel oil and n-alkanes (C18, C20, and C22) by a novel strain <i>Acinetobacter</i> sp. K-6 in unsaturated soil.	Chaudhary, D. K., Bajagain, R., Jeong, S. W., & Kim, J. (2020).
SA6	Bio-stimulation and bio-augmentation of native microbial community accelerated bioremediation of oil refinery sludge	Roy, A., Dutta, A., Pal, S., Gupta, A., Sarkar, J., Chatterjee, A., & Kazy, S. K. (2018).
SA7	Morphological, biochemical and molecular identification of petroleum hydrocarbons biodegradation bacteria isolated from oil polluted soil in Dhahran, Saudi Arabia	Al-Dhabaan, F. A. (2018).
SA8	Bioremediation potential of natural polyphenol rich green wastes: A review of current research and recommendations for future directions.	Kuppusamy, S., Thavamani, P., Megharaj, M., & Naidu, R. (2015).
SA9	Environmental Nanotechnology: Applications of Nanoparticles for Bioremediation	Bhandari G. (2018)
SA10	A Comprehensive Review of Aliphatic Hydrocarbon Biodegradation by Bacteria	Abbasi, F., Lockington, R., Mallavarapu, M., & Naidu, R. (2015).
SA11	Identification and overcome of limitations of weathered oil hydrocarbons bioremediation by an adapted <i>Bacillus sorensis</i> strain	Onalha, M., Al-Kaabi, N., Al-Ghouti, M., & Zouari, N. (2019).
SA12	Synergistic degradation of crude oil by indigenous bacterial consortium and exogenous fungus <i>Scedosporium boydii</i>	Yuan, X., Zhang, X., Chen, X., Kong, D., Liu, X., & Shen, S. (2018).
SA13	Microbial degradation of petroleum hydrocarbons	Varjani, S. J. (2017).
SA14	Abiotic factors controlling bioavailability and bioaccessibility of polycyclic aromatic hydrocarbons in soil: Putting together a bigger picture.	Yu, L., Duan, L., Naidu, R., & Semple, K. T. (2018).
SA15	A comprehensive guide of remediation technologies for oil contaminated soil - Present works and future directions.	Lim, M. W., Lau, E. V., & Poh, P. E. (2016).
SA16	A new look on factors affecting microbial degradation of petroleum hydrocarbon pollutants	Varjani, S. J., & Upasani, V. N. (2017).
SA17	Bioremediation of oily sludge polluted soil employing a novel strain of <i>Pseudomonas aeruginosa</i> and phytotoxicity of petroleum hydrocarbons for seed germination	Varjani, S., Upasani, V. N., & Pandey, A. (2020).

Fig 4. Scientific articles selected for the study.

3 Results and discussion

This section explains the results of the systematic literature review on the remediation of soil contaminated by crude oil through microbial degradation as an efficient method. The level of implementation of bioremediation in soil treatment is defined, the main aspects that influence the implementation of bioremediation are distinguished and the main stages of experimentation of the types of bioremediation that are used are determined, being crucial for future research by professionals and researchers in the environmental sector.

The search of the authors of these scientific articles is based on demonstrating the effectiveness of their bioremediation methods used using bacteria or a different agent, since each of these will grant a different appreciation regarding the use of the implementation.

3.1 Methodologies used in scientific articles

Some of the methodologies observed in the selected articles are the amplitude of the characterization of soil contaminated by crude oil, the selection of the microbial community in the soil treated under in vitro conditions and the compilation of the technologies used in the different scientific studies that will be part of the execution of the purpose of this study research. Initially, the contaminated soils are removed through a Dutch-type auger, at a depth of 0 to 25 cm deep, which are homogenized in labeled plastic buckets (PB) where they are placed to be taken to a laboratory for study. These PBs that contain the contaminated soils were thoroughly mixed and left to rest for a period of fourteen days; time necessary for the autochthonous microorganisms to acclimatize to the new soil condition [7].

While for [8] the subtracted contaminated soil sample it must be collected at a depth of 30 cm to reduce the pertinent intervention by animals or man. After being collected, these samples are kept in cold gel packs and adjusted to a temperature of 4°C, after arriving at the laboratory. The experiences of the laboratory tests are intended to remove the hydrocarbons present in the soil, observe the conditions of the soil contaminated by crude

oil (physicochemical-microbiological analysis of the contaminated soil), obtain a study of native bacterial strains that will be isolated and placed tested to measure its aggressive degrading capacity against hydrocarbons from crude oil in contaminated soil. These samples were transferred to the laboratory at rest, according to [9], the

samples must be analyzed physicochemically since it positively favors bioremediation as a traditional technique since factors such as: Bioavailability that alludes to the rate of degradation whose dependence it is the microbial metabolism that they have, it is related to the presence of water in required quantities that must be in contact with the contaminant; as well as also allow the growth of the

microbial community and the provision of electron acceptor such as oxygen that allows microbial respiration. In addition, the study of the molecular structure of the samples that are an important chemical factor since their study affects the analysis of their composition to determine their capacity for hydrocarbon biodegradation.

On the other hand, it must be taken into account that regarding the samples analyzed microbiologically speaking, their observation at the level of oxidation, degradation, transformation and the complete mineralization process of the contaminants adhered to the soil are important [9]; but, given the above, a factor must also be taken into account that is transcendental with what has been studied, which is the environmental factor of the soil; for which, the reviewed samples have a specific particularity, a temperature, a pH, oxygen, humidity and availability of nutrients, adhered to it [9].

Having carried out the study of the soil to be treated, the current state of the extracted sample was verified, so for [10] it is necessary to indicate the composition of the

crude oil that is at rest in the affected areas, being a negative impact on the environment. This composition can vary depending on the age of the affected oil field and its location, as well as the depth of the oil well and the polluting soil around it. Crude oil is divided into four broad fractions: Saturated (Aliphatic), Aromatic (Annulated Hydrocarbons), Resins and Asphaltenes. It is important to know this type of fractions because in the study of the extracted soil the percentage of each fraction is observed and based on this, verify what type of bacterial strain is its greatest degrading agent, as can be seen in Fig. 5.

Crude oil (Fractions)	Bacterial strain	References	
Aliphatics	Acinetobacter	Mittal y Singh (2009) y Foght (2008)	
	Alcanivorax sp.	Harayama y col. (2004) y Brooijmans et al. (2009)	
	Azoarcus sp.	Widdel y Rabus (2001)	
	Bacilo sp.	Ghazali et al. (2004) y Das y Mukherjee (2007)	
	Brevibacterium	Leahy y Colwell (1990)	
	Desulfosarcina sp.	Jaekel et al. (2013)	
	Desulfococcus sp.	Jaekel y col. (2013)	
	Marinobacter sp.	Yakimov et al. (2007)	
	Micrococcus sp.	Roy et al. (2002) y Ghazali et al. (2004)	
	Ochrobactrum sp.	Varjani et al. (2015)	
	Oleispira sp.	Harayama et al.(2004) y Brooijmans et al. (2009)	
	Pseudomonas sp.	Mittal y Singh (2009)	
	Rhodococcus sp.	Rocha et al. (2011). Sajna et al. (2015), Varjani et al.(2015)	
	Stenotrophomonas sp.	Varjani y Upasani (2016c)	
	Thalassolituus sp.	Abbasian y col. (2015)	
	Aspergilo sp.	Varjani et al. (2015)	
	Candida sp.	Brooijmans et al. (2009)	
Monocaromatics	Penicillium sp.	Wilkes y col. (2016) Leahy y Colwell (1990) Atlas (1981) Sajna y col. (2015)	
	Pseudozima sp.	Batista y col. (2006)	
	Acinetobacter sp.	Wilkes y col. (2016)	
	Archaeoglobus fulgidus	Janbandhu y Fulekar (2011)	
	Aromatoleum aromaticum	Widdel y Rabus (2001)	
	Bacilo sp.	Mittal	
	Halomonas sp.	Singh (2009) y Meckenstock et al. (2016)	
	Pseudomonas sp.	Leahy y Colwell (1990) y Salleh et al. (2003)	
	Rhodococcus sp.	Janbandhu y Fulekar (2011)	
	Sphingobacterium sp.		
	Polyaromatics	Achromobacter insolitus	Janbandhu y Fulekar (2011)
		Bacilo sp.	Mittal y Singh (2009)
		Cicloclasticus sp.	Harayama et al. (2004)
		Phanaerochaete chrysosporium	Salleh et al. (2003)
		Pseudomonas sp.	Widdel y Rabus (2001) y Meckenstock et al. (2016)
		Vibrio sp.	Widdel y Rabus (2001)
		Penicillium janthinellum	Boonchan et al. (2000)
Resins	Pseudomonas sp.	Leahy y Colwell (1990)	
	Family member	Chandra et al. (2013)	
	Vibrionaceae, Members of the		
	family Enterobacteriaceae, Moraxella sp.		

Fig 5. Components – fractions of crude oil which are degraded by bacterial strains.

In the process of the laboratory study, the isolation of bacteria that are attached to contaminated soils was developed. [11] explained that what is indicated to proceed in the phase of enrichment and isolation of hydrocarbon-degrading bacteria is the use of the selective enrichment technique and the Bushnell-Hass broth, which includes the increase in the strengthening and multiplication of the degrading microbial community, while the growth of other microorganisms that do not support the study is partially or totally inhibited and the crude oil of the contaminated sample is diluted. Said selected microbial community was gradually isolated and

placed in nutritive agars to give a control and maintenance routine to the pure bacterial strains. These pure bacterial strains were put under observation for their bacterial identification and characterization process; being decisive to choose if it is the most suitable degrading agent with the study and how much it can increase its performance with the respective stimulants. According to [12], a morphological characterization process must be developed in which its shape, color, transparency and what type of differential behavior it has in its colony must be verified, as well as the biochemical characterization process that is fundamental in the study, as shown in Fig 6. This allows various metabolic characteristics of bacterial strains to be observed and can be verified through rapid techniques such as the oxidase test, catalase test, urease test, indole test, Simmons citrate test, methyl red test (MR) and hydrolysis of gelatin.

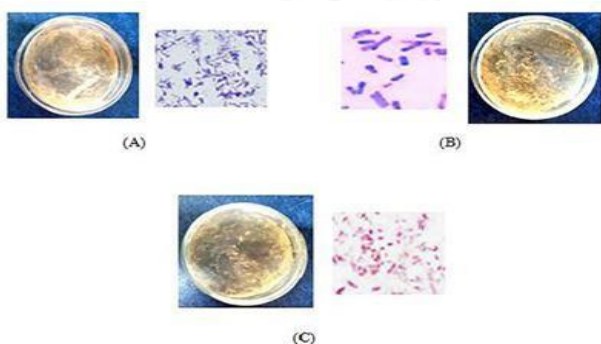


Fig. 6. Morphological characters and Gram stain for bacterial strains A, B and C isolated from Khurais oil. Font: Al-Dhabaan, F. A. (2018).

In various studies, it was possible to demonstrate the various bioremediation technologies used. For [8], in the field of bioremediation, biological alternatives were proposed that contributed to the oxidation, degradation, transformation and complete mineralization of the contaminating agents adhered to the soil. These bioremediation technologies are used according to ecosystem type criteria that include climatic variables in the affected areas at the water table, type and structure of soil, concentration of the type of hydrocarbon, environmental impact, investment budget, among others.

The technologies used by professionals in the environmental sector are mentioned below.

In this study, given the selected scientific articles, bioaugmentation and biostimulation were chosen as the most appropriate bioremediation technologies to be used in the soil to be treated. As can be seen in Fig 7, it explains [8] that in developing his experimentation with four bioremediation techniques (Natural Attenuation, Bioaugmentation, Biostimulation and Bioaugmentation + Biostimulation) and separating his samples with different nutrients such as selected native Bacteria (BA), Nitrate (N), Phosphorus (P) or combined (NP), found that one of the samples had a significant performance; as a result of the practice of the Bioaugmentation + biostimulation technique (BA + NP) through which an increase of 75% was observed compared to the rest of the samples that

have between 35% and 63% of hydrocarbon degradation, being the largest with respect to the rest of the proposed samples.

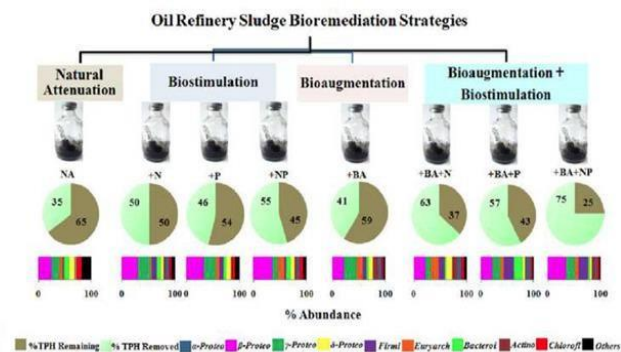


Fig. 7. Comparison of the methods used for the experimentation of the remediation of contaminated soil at the laboratory level. Font: Roy, A. et al. (2018).

According to the above figure, these authors considered that this improvement is substantial and stable due to the increase in the yield of the *Bacillus*, *Archrobacter*, *Rhodobacter* and *Pseudomonas* strain bacteria used, adding nutrients such as NP that, in effect, promoted the activation of sulfate reducers, the generation of fermentative abundances, increased yield of hydrocarbon degraders and high activity of methanogenic populations that are within the contaminated soil microbiome [8].

On the other hand, according to [13], who developed a pyro-sequencing study to verify changes in bacterial diversity and the proportions of their populations in soils contaminated by crude oil. He argues that this type of treatment with various concentrations of crude oil, showed severe effects on bacterial communities and their biodiversity in soils, clear detail was observed in several bacterial communities, such as Fibrobacterales, Thermoanaerobacterales, Nautiliales, Enterobacterales, Thermotogales, Ustilaginales and Plocamiales that had a high susceptibility in the treatment and disappeared in the contaminated soils with low concentrations of crude oil of 0.5% and 2.5%. Being able to show that, not only should an appropriate selection technique be developed for the bacterial communities to later be subjected to bioaugmentation, but also biostimulation should be added to maximize the performance of the bacterial communities so that they can resist these concentrations, making the degradability and its ability to resist high levels of contaminants greater.

After synthesizing the importance of bioaugmentation + biostimulation in the research and with the intention of corroborating the effectiveness of this treatment [14], he carried out the germination of a *Vigna radiata* plant, which under normal conditions has a germination rate of 95.23% at fifth day of experimentation, while in the case of soil contaminated by crude oil, it was observed that its germination rate is 4.76% on the tenth day of experimentation. Having evidenced this significant difference in the germination rate, the bacterial

inoculation supplementation was developed together with other nutrients that help the *Vigna radiata* seeds to have a germination rate of 80.95% on the fifth day of experimentation, considering the good progressive and rapid growth, demonstrating that the contaminated land of the extracted sample has managed to largely eliminate the concentration of crude oil present, for which this treated land became fertile again, having great relevance in agricultural and environmental sustainability, providing a way to agricultural application on land contaminated by crude oil after the bioremediation process.

Finally, in the present investigation carried out based on the selected scientific articles, it was shown an effective experimental process with respect to their studies, specifically, in biostimulation + bioaugmentation techniques, having as a key point the sum of these two techniques as a more complete method for bioremediation in situ, being viable as an associated technology with a great impact on the environment in the areas surrounding the area contaminated by crude oil.

4 Conclusions

From the systematic review of the literature, it was possible to verify that the remediation of soils contaminated by crude oil has been a relevant research topic in the last six years. As a result of these investigations, it has been possible to find and verify the effectiveness of the use of bacterial strains in the recovery and restoration of soils contaminated by crude oil.

When the compilation and revision of the scientific articles was carried out, it was possible to appreciate that a large part of these reflected positive experiences, obtained in the laboratory. Leaving in evidence that although bioremediation applications were developed using various tools with optimal results in their experimentation; In reality, they had a great gap when putting them into practice in the field or affected area, not being able to obtain the expected results because the conditions of the variables that condition the process of degradation of the contaminated soil are not the same.

However, the importance of laboratory-scale studies is not denied, since their development is essential since it can minimize the various problems associated with their application of In-Situ bioremediation and achieve a significant improvement in the use of the method [15].

On the other hand, the behavior of the crude oil fractions is unique to each contaminated area as well as the impact generated in them; that is why exhaustive evaluations are carried out, to determine what would be the type of appropriate technique with which soil remediation will be addressed [16]. Instead, the influence of abiotic factors in direct relation to soil structure must be understood intrinsically. Defining the series of parameters involved is a complex task that makes it difficult to develop a global procedural method regarding the various factors that control the bioavailability and bioaccessibility of crude oil in contaminated soil [17].

Each bioremediation technology used has its own characteristics and the cleaning methods applied will not

be established as universal methods but as alternatives for the bioremediation of soils contaminated by crude oil [16]. Based on this, the mixed design of bioremediation technologies is proposed that allows the integration of two or more bioremediation technologies given that they have a higher performance and effectiveness in soil restoration, as in the case of Bioaugmentation + Biostimulation that were used in other investigations [18].

As part of the research, new proposals were observed to cover the remediation of contaminated soils, one of them being nanoremediation, which is an approach under development and has a promising perspective in improving bioremediation [19], incorporating the different types of food, agro-industrial and tree waste materials; as important sources of polyphenols in the successful removal of environmental pollutants [20]; and as a last proposal, genetic engineering, which proposes the genetic modification of the microorganisms or organisms used, although the application of this proposal is controversial and with little acceptance, due to the fact that the real impact that these microorganisms or organisms could have on the the ecosystem.

Finally, the great challenge in the future for researchers is to achieve a broader coverage of their knowledge regarding the microbial populations that degrade crude oil hydrocarbons [21], deeply understanding that symbiotic relationships and their metabolizing pathways implant in the complicated system of the contaminated soil. The proposal of this will help to expand and extrapolate the knowledge obtained in pilot scale experimentations in large complex field systems.

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