Improvement of Bacterial Activity for the Depollution of Contaminated Soil by Diesel Oil

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ABSTRACT: *Some human activities cause soil and groundwater pollution. Physical and chemical processes are used to eliminate or reduce this contamination. However, these techniques are very expensive and can be very invasive to ecosystems. Bioremediation is a remediation process that uses bacteria's metabolic capacity to degrade organic pollutants. Certain parameters, however, have a significant impact on the development of the bacteria, namely the level of oxygen and the nutrient content, particularly nitrogen and phosphorus (N and P). In this study, the effect of these two parameters on bacterial reproduction and decontamination power on soil polluted by diesel oil at 10 g/kg was investigated. Bubble column bioreactors have been used; each bioreactor was filled with polluted soil. The experiments were carried out in two configurations, A and B, to characterize separately the biodegradation rate generated solely by bacterial activity and the removal rate generated by aeration. In the first case, bio-stimulation was used to increase the bacterial flora, while in the second, the bacterial flora was neutralized by using* $HgCl_2$*. The contaminated soil was amended using* NH_4Cl*, and* KH_2PO_4*, salts according to C/N/P molar ratios of 100/10/1, 100/5/1, 100/25/1, 10/10/3, and 100/10/0.33. The results showed a significant relationship between airflow rate, C/N/P molar ratio, and diesel oil removal on the one hand, and biomass growth on the other. After 26 days, the removal rates were 58, 70, 79, 78, and 97% for 0.25, 0.5, 1, 1.5, and 2 L/min, respectively, for a C/N/P molar ratio of 100/10/1. Furthermore, after 12 days, the best biodegradation rate was 36% with an airflow rate of 1 L/min and a C/N/P molar ratio of 100/10/1. It would be interesting to continue this research to determine the best conditions for increasing the rate of biodegradation.*

KEYWORDS: *Diesel oil, pollution, bioremediation, nutrients, aeration, microorganisms.*

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INTRODUCTION

The environmental problems are often based on the generation of different kinds of pollutants that arise from industrialization, causing untold damage to all spheres of life. In this regard, the intensive use of petroleum products, as well as the expansion of activities involving petroleum resources, raise major concerns about water and soil pollution from accidental spills [1]. Aromatic solvents, liquid fuels, and polycyclic aromatic hydrocarbons (PAHs) are the main compounds often detected in polluted areas. PAHs can be regarded as substances of main concern in oil spills because of their toxicity to human health, groundwater, and soil ecological systems [2], which need appropriate remediation methods.

The most common treatment techniques are costly and can generate toxic substances in the environment. To remediate and manage polluted sites, safe and cost-effective strategies are required. Among available remediation alternatives, the bioremediation is a natural method that allows one to convert pollutants into inorganic compounds, nutrients, and cell biomass [3] or remove the adverse effects of pollutants depending on the capacity of the microorganisms involved or their products [4]. A microbial consortium isolated from polluted soil has the potential to be used in the clean-up processes of polluted soil with hydrocarbons, with 93.84% of the diesel consumed in the bubble-column reactor after 15 days of culture [5]. This method offers a promising approach in terms of profitability and respect for the environment to clean up soil and water contaminated by hydrocarbons, in particular if they are applied in situ [6]. TPH removal efficiency of 45.4% (w/w) in microcosms confirmed the potential of indigenous microorganisms for bioremediation of the aged oil-based drilling waste [7].

This technique could be improved by optimizing the environmental conditions such as temperature, pH, soil moisture, oxygen, temperature, and mineral nutrients, which affect both the bacterial growth and activity [8].

Oil pollution can cause a nitrogen deficiency of nitrogen in comparison to carbon, which is a limiting factor [9]. To enhance the microorganism's activity and improve biodegradation efficiency, it is important to ensure a continuous supply of oxygen and nutrients [10-11]. Some researchers have added food residues as nutrients in the case of bioremediation of petroleum hydrocarbons [12].

Oxygen is also a limiting factor, which affects the efficacy of the bioremediation methods, particularly in the breakdown of fuels under aerated conditions. To enhance the bioremediation process, the most common methods consist of using titling, forced aeration, and the addition of bulking agents [13,14]. *Meyer et al*. point out that the presence of easily oxidizable fatty acids and the absence of aromatic compounds improves biodiesel remediation [15]. Manual aeration and the use of two microbial autochthonous strains for the bioremediation of diesel-contaminated soil and soil-sawdust mixture improve diesel oil elimination, which reached yields of 79 and 93.53%, respectively, after 45 days of treatment [16]. Besides, the use of polluted sites as sources of mixed microbial communities for hydrocarbon degradation appears promising since it can degrade the substrate beyond the yield of 80% after 40 h of treatment [17]. *Marchand* C. *and al.* investigated the potential for PAH biodegradation using 95 bacteria isolated from a former petrochemical plant. Three of the strains, Rhodococcus sp., Trichoderma tomentosum, and Fusarium oxysporum, significantly degraded all PAH compounds [18].

Soils contaminated with a mixture of three common hydrocarbons were treated using consortiums of microbes isolated from 3 different locations; the biodegradation rate was almost 100% after 30 days [19]. The degradation rate of diesel oil in a composting process for contaminated soil has been performed by comparing three intermittent aerations with a continuous mode. They have found that the most active degradation of diesel oil and normal alkanes occurs during intermittent aeration [20]. Table 1 compares the pollutant removal rates achieved in this study with the other methods mentioned in this paper.

The aim of this study is to investigate the potential of bioremediation techniques in treating diesel oil-polluted sandy soil using biostimulation and bioaugmentation approaches. The optimal physiological conditions and the effect of specific inorganic N and P nutrient additions as well as oxygen have been assessed to highlight the diesel oil biodegradation rate. Moreover, the film thickness and the characteristic time of external diffusion have been estimated.

EXPERIMENTAL SECTION

Technical analysis

Quantification of oil pollution levels was performed by the gravimetric method and gas chromatography (GC), (Agilent 7890, FID detector). Both analysis techniques

Table 1: Values of the pollutant removal rates

Methods	Pollutant	Operating conditions	Duration (day)	Removal Rates (%)
The present study	Gasoil	Aeration and nutrients	26	97
[5]	Gasoil	Bubble-column reactor	15	93.84
[7]	TPH	Indigenous microorganisms in microcosm.		45.4
[16]	Gasoil	Two microbial autochthonous strains with manual aeration	45	79
[19]	Mixture of hydrocarbons	Consortia of microbes isolated from different locations	30	100
[20]	Mixture of hydrocarbons	Intermittent aeration. Continuous aeration	30	96.5 98.1

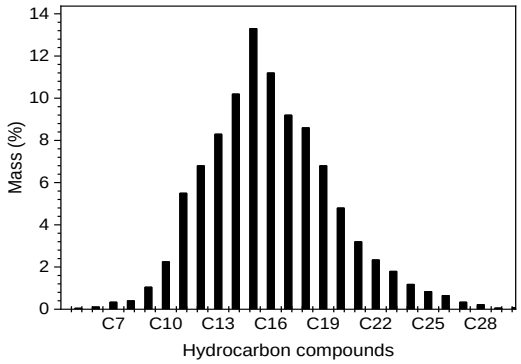

Fig. 1: Diesel composition

provided quite similar quantifications regarding the diesel oil degradation, as previously reported by *Varjani* and *Upasani* [21]. 5 g of contaminated soil sample was mixed and crushed with extra-pure Na_2SO_4 at a mass ratio of 4/5 and extracted with dichloromethane (DCM) by sonication for 15 min. After centrifugation, the extract was removed, and the process was repeated twice, the first with 30 mL and the second with 15 mL of DCM; the total extract was concentrated at 40°C (rotary evaporation) and then weighed. The diesel oil content was adjusted to dry weight; note that due to its composition, diesel oil does not contain bitumen. The reproducibility of diesel oil measurement was determined by duplicating sampling, and the average result was reported. To count the heterotrophic microbial population, 1 g of soil was serially diluted and spread on nutrient agar. Plates were incubated for 24-48 h at 30 °C prior to counting Colony-Forming Units (CFU), and biomass was expressed on dry matter. Phosphorous (ISO 6878), nitrates (ISO 5667-3), nitrites (NFT 90 013), and ammoniacal nitrogen (ISO 7150-1) contents were determined by means of a UV spectrophotometer, the Jasco V-530, at the wavelengths of 880, 420, 543, and 655 nm, respectively.

The soil pH was determined using a pH meter (multi-parameter analyzer Consort C3010) with a soil-deionised water ratio of 1/5 (w/v) after stirring for 1 hour.

Soil characteristics

The soil material consisted of sandy soil collected from a seaside beach in Bordj El Kiffane, 15 km east of Algiers, located at ten meters near a manhole. This choice is based on the microbial communities that may be used for bioremediation of soil polluted with diesel oil. Scientific research carried out with the same soil has indicated that microscopic observations of the isolated strains show that there are both types of bacteria in our soil: gram-positive and gram-negative bacteria [22]. The most frequent active bacteria in the natural environment are: Pseudomonas, Acinetobacter, Flavobacterium, Mycobacterium, Brevibacterium, Corynebacterium, and Arthorbacter [23].

The physico-chemical characteristics and biomass populations of this sandy soil are given in Table 2. It can be seen that the sandy soil is low in organic carbon (0.17%), while the inorganic carbon content is 43.2 mg/g. Also, the selected soil remains alkaline and poor in mineral elements such as nitrogen and phosphorous, which are equal to 2.27 10^{-3} and 1.84 10^{-5} mg/g, which correspond to C/N and C/P molar ratios of 606 and 1.68 10^5. The soil has a pH of 8.35, measured in contact with the atmosphere indicating that the pH is controlled by calcocarbonic equilibria. Diesel oil composition is given in Fig. 1; it is a complex mixture of alkanes and aromatic compounds that can have carbon chain lengths ranging from C_6 to C_{29} with 29-30% of alkanes between C_{10} and C_{24} [22]. Some researchers have emphasized that diesels that include n-alkanes can be fully degraded, while heavy molecules can be significantly reduced at lower ratios [24].

Table 2: Physical and chemical sandy soil characteristics

Characteristics	Value	Characteristics	Value
d_h (µm)	401	C/P (Molar ratio)	$2.37.10^5$
ρ_s (kg/m^3)	2604	Humidity rate (%)	0.61
CO (mg/g)	1.7	pH	8.35
C (mg/g)	43.2	Biomass (CFU/g)	$3.14.10^4$
N (mg/g)	$2.27.10^{-3}$	Clay (%)	0
P (mg/g)	$1.84.10^{-5}$	Coarse sand (%)	98
C/N (Molar ratio)	870	Fine sand (%)	1.49

Soil preparation

A mass of 15 kg of soil has been dried at a temperature of 19 °C for 72 h. Sandy soil samples were homogenized, using the standard NF: X 312-412 and sieved with a sieve of 800 µm pore diameter. The structure of sandy soil has a great effect on the bioremediation of polluted soils [25], it has been reported that a dense structure of soil often limits the oxygen transport into the bacterial flora.

The number of bacteria is a determining factor for hydrocarbon degradation. Bacteria cells in the soil sample approach 3.14×10^4 CFU/g, which seems insufficient to degrade the mixture of hydrocarbons. The effectiveness of bioremediation is related to microbial growth and how it can be enriched and maintained in the environment [26]. A value of 10^6 CFU/g is often recommended as a minimum of bacterial population [27]. Microbial flora needs mineral elements for its growth, especially nitrogen. These elements are necessary for the formation of cellular constituents during the multiplication of microorganisms; thus, an enrichment of the sandy soil by phosphorous and nitrogenous sources seems essential.

Sandy soil biostimulation

In this study, the sandy soil was biostimulated to reach a total biomass value of 10^6 CFU/g. A sample of 4 kg of the sieved sandy soil was polluted with 10 g of diesel oil: the diesel oil was blended with 500 mL of water under magnetic stirring (400 rpm) at 30 °C. The water-diesel oil mixture was sprayed into the sandy soil and mixed for 10 minutes. To amend the soil, 500 mL of a mineral solution as nitrogen and phosphorus sources containing 2 g of NH_4Cl, 1 g of KNO_3, and 0.5 g of KH_2PO_4 was added to the sandy soil and thoroughly mixed. Then, the soil sample was incubated at 27 °C for one week. The soil has been watered by 400 to 600 mL to keep the humidity rate at 15%. Thereafter, the bacterial population has reached a value of 10^6 CFU/g.

Preparation of sandy soil samples for tests

After biostimulation, the sandy soil sample was analyzed to determine residual diesel oil concentration, nitrogen and phosphorus contents, and humidity rate. Before each experiment, the concentration of the diesel oil was adjusted to 10 g/kg, the humidity rate to 15%,and the nitrogen and phosphorus contents were readjusted according to C / N / P ratios equal to 100/10/1, 100/5/1, 100/25/1, 10/10/3, and 100/10/0.33; for this purpose, mineral solutions of ammonium nitrate and potassium phosphate were selected.

Alexander et al. recommended a ratio of C / N / P / K = 100/10/1/2.5 for bioremediation studies [28]. Several authors have used different ratios 120/10/1, 60/2/1, and 150/10/3 [29-30-31].

Experimental Setup

The experimental setup consists of several cylindrical columns (reactors) of 500 mm height and 80 mm inner diameter (Fig. 2). Each reactor was filled with a sandy soil sample weighing 1200 to 1400 g. Air was injected in upflow mode by a compressor (FIAC, IP 44 I. CL) and passes through the soil bed, which has an initial porosity of 0.387 and a height of 190 to 210 mm. Air flow rates were varied from 0.25 to 2.0 L/min and measured by a flowmeter (Techfluid 2150). The air was introduced into each reactor through a 5 mm internal diameter orifice and passed through a Raschig rings layer of 7 cm in height.

The water percolates by gravity by means of dosing pumps (ISMATEC Model ISM 1076A) to reach the soil humidity of 15 %. Each reactor was provided with 4 openings (sampling taps) of 8 mm diameter for the withdrawal of sand samples. The distance between each opening was about 50 mm.

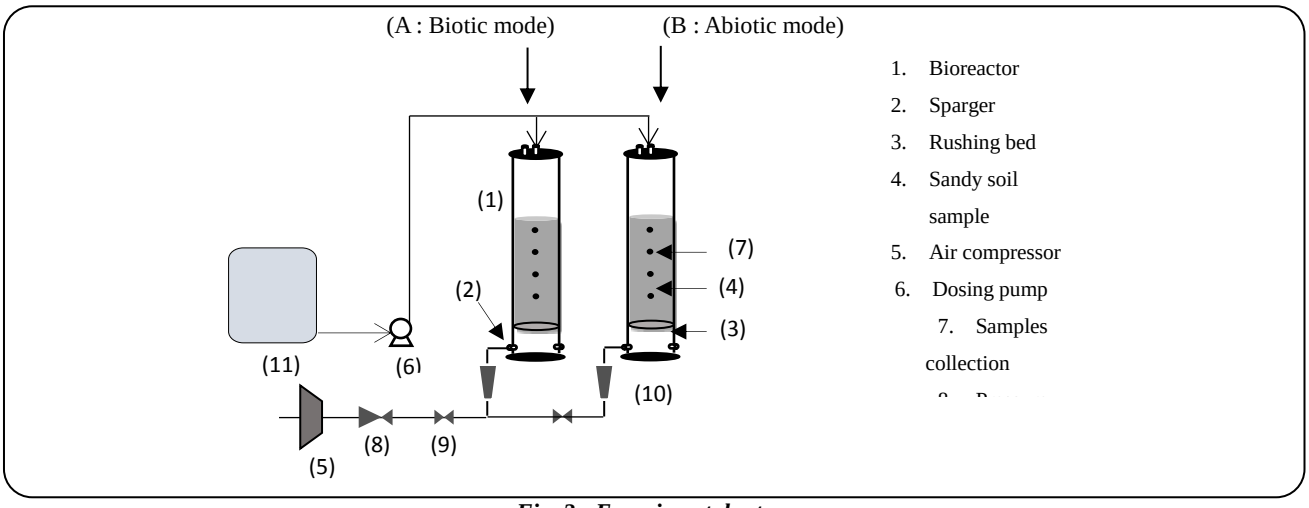

Fig. 2: Experimental setup.

To assess the effect of air with respect to microorganisms on diesel oil degradation, the experiments have been carried out under biotic mode with an amended soil including microorganisms and under abiotic mode, with a sterilized soil and $HgCl_2$ solution (0.5%) to assess diesel oil losses. In comparison, a biodegradation in natural attenuation mode with no air flow rate and a constant humidity rate of 15% was also performed. All tests were performed at 20°C. Note that experiments have been run at velocities below the minimum fluidization velocity, V_{mf} = 27 cm/s, i.e., in a fixed bed reactor.

Diesel removal and biodegradation ratios

The total removal ratio (T_R) leads to an assessment of the removal of the diesel oil in the biotic mode, which is generated by the combined effects of bacterial activity and mass transfer generated by aeration.

The degradation (T_{abiot}) ratio determines the diesel oil removal in abiotic mode due only to air entrainment, where air is introduced in the bed to diffuse toward the interface.

The T_{biod} ratio provides information about diesel oil uptake by the biodegradation process triggered solely by bacterial activity. These ratios can be calculated using the following equations:

$$T_R=(C_o-C_{biot})/C_0 \quad (1)$$

$$T_{abiot}=(C_0-C_{abiot})/C_0 \quad (2)$$

$$T_{biod}=(C_{abiot}-C_{biot})/C_0 \quad (3)$$

where:

C_0 : Initial diesel concentration (g/kg);

C_{biot} : Diesel concentration over time in biotic mode (g/kg);

C_{abiot}: Diesel concentration over time in abiotic mode (g/kg).

Kinetics of diesel oil degradation

In biotic mode, petroleum-hydrocarbon degradation often follows pseudo-first-order model [32-33]. The mass balance in a homogenous closed reactor gives:

$$V=-\frac{dC(t)}{dt}=k.C(t) \quad (4)$$

The integration of equation (4) leads to the following equation:

$$\text{Ln}\ \frac{C(t)-C_{lim}}{C_0-C_{lim}} = -kt \quad (5)$$

Where:

C(t) is the diesel oil concentration over time (g/kg), *k* the rate constant of diesel oil removal (/day) and C_{lim}, the limit concentration.

Diesel oil biodegradation induced only by bacterial activity is characterized by the kinetic rate constant k_{biod}, determined by considering, the residual concentration, C_{biod} which is replaced in Eq.5:

$$C_{biod}= C_0-(C_{abiot}-C_{biot}) \quad (6)$$

Where:

C_{biod} is the residual diesel concentration over time (g/kg);

Diffusion of light diesel oil fractions in air and film thickness

The diffusion coefficient is the ratio between the molar flux of species that diffuse in a gaseous mixture and the concentration gradient according to Fick's law. This can be applied to light diesel oil fractions in a mixture

of diesel oil and air. Several correlations are available in the literature, among which the kinetic theory of gases has been suggested to account for attraction and repulsion phenomena. The diffusion coefficients can be estimated using the Lennard-Jones parameter [34].

$$D_{fuel\text{-}air}=0{,}0018583\ T^{\frac{3}{2}}\ \frac{\left[\frac{1}{M_{fuel}}+\frac{1}{M_{air}}\right]^{\frac{1}{2}}}{P\,(\Omega_{fuel\text{-}air})\sigma_{fuel\text{-}air}^{2}} \quad (7)$$

where :

$D_{fuel\text{-}air}$: Diffusion coefficient, $\frac{cm^2}{s}$

M_{fuel}, M_{air} : Molecular weights of fuel and air

T: Temperature, K

P: Pressure, bar

$\sigma_{fuel\text{-}air}$: Lennard-Jones parameter, Å

$\Omega_{fuel\text{-}air}$: Diffusion collision intégral, dimensionless

The Lennard-Jones parameter can be estimated by the following equation:

$$\sigma_{fuel-air}=\frac{1}{2}\,(\sigma_{fuel}+\sigma_{air})=\frac{1}{2}(\sigma_{fuel}+\sigma_{air}) \quad (8)$$

Diffusion collision integral can be estimated by :

$$\frac{K\,T}{\varepsilon_{fuel\ air}}=T\sqrt{\frac{K}{\varepsilon_{fuel}}\frac{K}{\varepsilon_{air}}} \quad (9)$$

The Schmidt number is the dimensionless ratio between the momentum and the mass diffusivities:

$$Sc=\frac{\mu}{\rho\ D_{fuel\text{-}air}} \quad (10)$$

The Reynolds number is given by the following equation :

$$Re=\frac{\rho\ u\ d_p}{\mu} \quad (11)$$

The Sherwood number is correlated by :

$$Sh = 2 + 1.8\ Re^{\frac{1}{2}}\ Sc^{\frac{1}{3}} \quad (12)$$

Film thickness can be estimated by :

$$\delta_{film}=\frac{d_p}{Sh} \quad (13)$$

Characteristic time of external diffusion :

$$t_{ext.diff.}=\frac{d_p\ \delta_{film}}{D_{fuel\text{-}air}} \quad (14)$$

RESULTS AND DISCUSSION

Effect of aeration on diesel oil degradation

The diesel profiles of evolution in biotic and abiotic modes for different airflow rates and a C/N/P = 100/10/1 ratio are given in Fig. 3. It can be seen that the diesel oil removal in biotic mode exhibits an exponential shape under different airflow rates (Fig. 3A). This figure shows a significant decrease in diesel oil concentration during the first week, which drops from 10 to 7.80, 6.12, 5.30, 3.70, 3.77, and 3.22 g/kg for the natural attenuation and air flow rates of 0.25, 0.50, 1, 1.5, and 2 L/min, respectively. The corresponding removal rates, T_R, reached are 22, 39, 47, 63, 62, and 69 %, respectively. At the beginning, oxygen is used as an electron acceptor by the aerobic bacteria to oxidize hydrocarbon components [35]. This means that in a biotic process, the injection of airflow rate throughout the bed increases the diesel oil efficiency due to the combined effect of aeration and indigenous bacteria activity; improving mineralization and biomass production [22]. The maximum specific bacteria growth reached 1.8510^{10} (/day) for 1 L/min (Table 2). The same observations have been previously reported by *Fotinich et al.* [36]. Intermittent aeration mode (1 h aeration and 3 h rest) enhances a mixture of hydrocarbon degradation rates to 96.5% after 30 days of incubation [20]. Continuous aeration mode improves slightly hydrocarbons removal (98.1%). In the absence of ventilation systems, certain researchers have obtained only 27% and 60% after 7 and 17 days of soil treatment, respectively [37]. The incomplete bioremediation process may be due to a decrease in the bacterial population numbers caused by a lack of nutrients and an excess of air, which inhibit bacteria growth. It can also be seen that the diesel oil removal is significantly lower in the abiotic mode compared to the biotic one, due to the driving force for mass transfer and evaporation phenomena that occurred during the same period (Fig.3B). The abiotic loss of diesel oil by volatilization is estimated at 10% and can reach 12% over time [38].

Air injection into contaminated soil in an abiotic process does not improve diesel oil degradation compared to the combined effect of aeration and bacterial activity in the biotic mode due to the decrease in diesel oil solubility and oxygen availability to bacteria. The hydrocarbons solubility is still the critical issue in the bioremediation process [39]. It is therefore essential that oxygen supply and nutrients inside the bubble column reactor be optimized.

Note that the differences in the diesel oil degradation rates observed between the biotic and abiotic processes

Table 3. Degradation rates in the case of biotic, abiotic and control modes.

Airflow (L/min)	0 (NA)	0.25	0.50	1.00	1.50	2.00
μ_{max} (/day)	$3.44.10^{6}$	$9.92.10^{5}$	$1.24.10^{7}$	1.8510^{10}	$2.8.10^{9}$	$2.75.10^{7}$
T_R (%)	33	58	70	80	77	97
T_{abiot} (%)	-	41	53	54	64	87
T_{biod} (%)	-	18	17	36	10	9

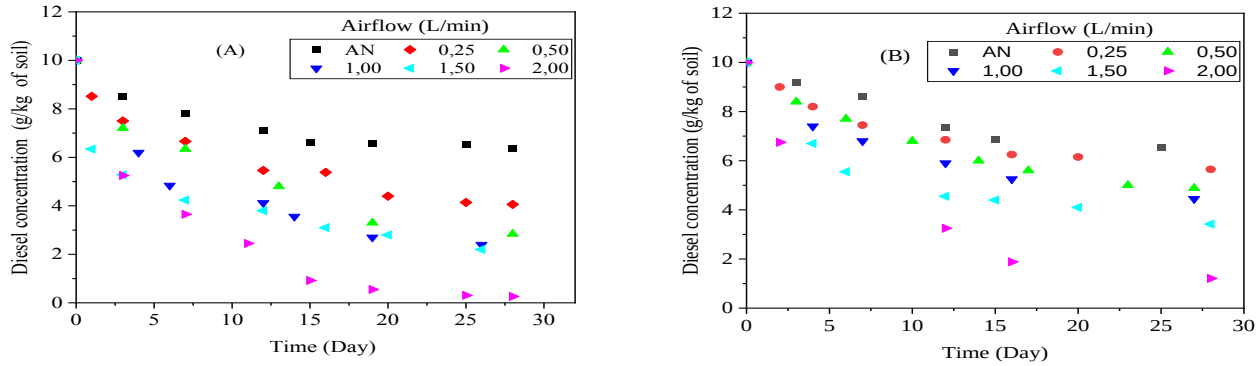

Fig. 3: Evolution of diesel concentration for different airflow rates (g/kg of soil). (A) Biotic mode. (B) Abiotic mode. C/N/P = 100/10/1.

highlight the biological oxidation of diesel in the biotic mode, while the entrainment phenomenon remains the main driving process in the abiotic mode (Table 2, Fig. 3).

The diesel oil concentration continues to decrease in both modes until it reaches stability at day 21. At the 25th day, the final concentrations, C_{lim}, are 4.15, 3.0, 1.94, 2.28, and 0.31 g/kg for the air flow rates of 0.25, 0.50, 1, 1.50, and 2 L/min in the biotic mode, which correspond to the total removal rates of 58, 70, 80, 77, and 97 %. In abiotic mode, the final removal rates reached on the 25th day are 41, 53, 54, 64, and 87%, respectively. This can be explained by nutrients depletion and/or degradation of the most recalcitrant hydrocarbon compounds [22]. These findings are slightly higher than those obtained by *Najirad et al.* [40]; these authors have tested indigenous bacteria from contaminated soil coming from a refinery, and they reached a hydrocarbon mixture degradation of 93% after 45 days of treatment. Diesel oil degradation depends mainly on the optimum conditions for bacteria activity and respiration in the contaminated sandy soil. It is important to know that mass transfer phenomena influence diesel oil degradation in sandy soil, resulting in a decrease in hydrophobic pollutants sorption in the soil matrix, which can improve the rate and extent of biodegradation [41]. In this study, the optimal diesel oil degradation is 97% for an airflow rate of 2 L/min. Some authors tested the ability of Klebsiella pneumoniae ATCC13883 and additional sources such as carbon, nitrogen, and surfactants to assess petroleum degradation. The highest yield reached is 66.5% in the case of petroleum concentrations of 1% v/v [42]. The biodegradation of PAH (phenanthrene) was studied using indigenous bacteria isolated from two petroleum-contaminated sites; results revealed a significant relationship between concentration and type of microbial consortium with the removal efficiency [43].

This strain can efficiently metabolize C_{10}-C_{20} alkane hydrocarbons in petroleum due to its complex enzyme system.

Effect of aeration on bacterial growth

Bacterial growth in the soil in biotic mode is given in Fig. 4. It can be seen that for different airflow rates, the bacterial population increases quickly during the first week. It seems that this is an active phase in which the bacteria number strongly increases to the highest values of 1.40×10^{7}, 1.20×10^{8}, 5.40×10^{10}, 9.50×10^{9}, 8.20×10^{8} and 1.60×10^{7} CFU/g for the air flow rates of 0.25, 0.50, 1.00, 1.50, and 2 L/min and for the Natural Attenuation (NA), respectively. These results show that the aerobic mode led to an increase in the population number of bacteria compared to untreated soil (NA). This is in line with the rates of diesel oil degradation observed in the soil sample.

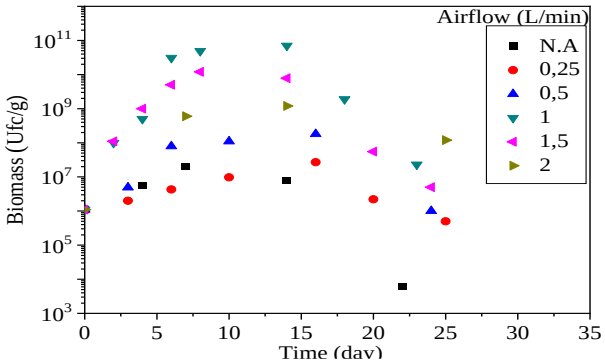

Fig. 4: Evolution of bacterial growth for different airflow rates, C/N/P = 100/10/1.

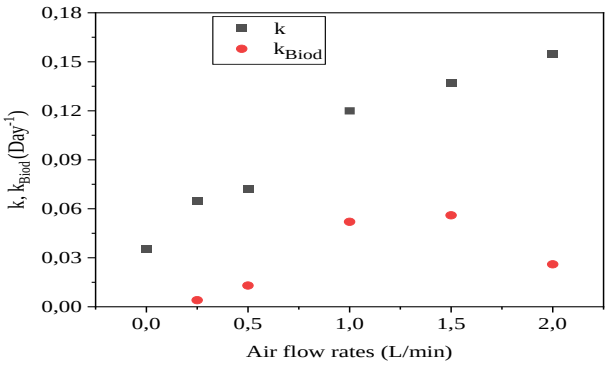

Fig. 5: Influence of the airflow rates on the kinetic rate constant of the diesel removal, k, and on the diesel biodegradation, k_{biod}. C/N/P = 100/10/1.

The aeration and nutrients enhance the growth of microorganisms. This active phase is followed by a pseudo-stability phase in which the number of bacteria cells remains almost constant until the 15th day. Between the 15th and the 25th day, the number of bacteria cells decreases quickly. During this period, the total removal rate, T_R has increased very slightly from 34 to 34.5 % for the natural attenuation and from 90 to 97%, for the airflow rate of 2 L/min. This period corresponds to the decline phase of the microorganism's growth, in which the bacteria may begin to biodegrade recalcitrant compounds; as a result, their potential degradation is reduced [44]. In such a case, the decrease in the diesel oil concentration is due to the driving phenomenon caused by the airflow rates and the indigenous microorganisms existing in the soil. The latter exhibit a special metabolism to break down the hydrocarbon compounds in diesel and to avoid cells mortality by bypassing certain metabolic reactions [45-46]. Often, the change in the bacterial numbers is indicative of a stimulated biodegradation process [47]. Note that the adaptation phase does not appear since the soil is amended by bio-stimulation using diesel oil as a single source of carbon and energy. The highest number of bacteria cells reached is 5.40×1010 CFU/g after 7 days for an airflow rate of 1 L/min, corresponding to a bacterial growth rate μmax of 1.851010 (/day). For higher rates, the reached values are lower: 9.50×109 and 8.20×108 CFU/g for 1.5 and 2 L/min, respectively. It seems that an excess of air inhibits bacterial growth; this is probably due to the reduction of the bacterial film caused by the turbulence generated by the high airflow rates. Excess turbulence inhibited microbial growth and metabolism [48].

Film thickness

A Lennard-Jones parameter is given by:

$$\sigma_{fuel\text{-}air} = \frac{1}{2}(\sigma_{fuel} + \sigma_{air}) = 2.87 \text{ Å}$$

Diffusion collision intégral is estimated by:

$$\frac{K\,T}{\varepsilon_{fuel\ air}} = T\sqrt{\frac{K}{\varepsilon_{fuel}}\frac{K}{\varepsilon_{air}}} = 1.507$$

$$\Omega_{fuel\text{-}air} = 1.196$$

Diffusion coefficient :

$$D_{fuel\text{-}air} = 0.192\ 10^{-4}\ m^2/s$$

The Schmidt number is the dimensionless ratio between the momentum and the mass diffusivities:

$$Sc = \frac{\mu}{\rho\ D_{fuel\text{-}air}} = 0.819$$

The Reynolds number is given by the following equation:

$$Re = \frac{\rho\ u\ d_p}{\mu} = 0.0763$$

The Sherwood number is correlated by:

$$Sh = 2 + 1.8\ Re^{\frac{1}{2}}\ Sc^{\frac{1}{3}} = 2.45$$

Film thickness can be estimated by:

$$\delta_{film} = \frac{d_p}{Sh} = 1.64\ 10^{-4}\ m$$

The film thickness and the characteristic time for each flow rate are given in Table 4. It can be seen that the increase in flow rate leads to an increase in both the Reynolds and Sherwood numbers, while the film thickness and the characteristic time decrease. Moreover,

Table 4: Film thickness and characteristic time

Air flow rate (L/min)	U (m/s)	Re	**Sh**	δ_{film}(μm)	$t_{ext.diff}$ (s)
0.25	$8.33.\ 10^{-4}$	0.022	2.248	178	0.0037
0.50	$1.66.\ 10^{-3}$	0.043	2.346	171	0.0036
1.00	0.003	0.086	2.490	161	0.0034
1.50	0.005	1.30	3.907	102.6	0.0021

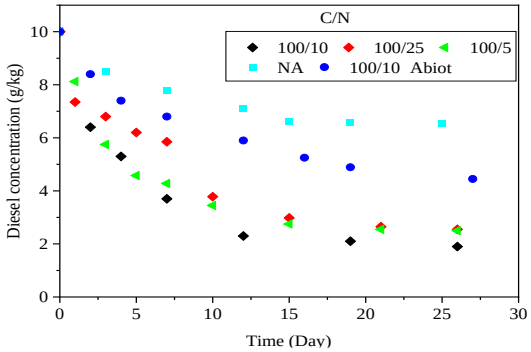

Fig. 6: Evolution of the diesel concentration for different molar ratios
C/N in biotic mode. C/P = 100/1. Airflow = 1L/min

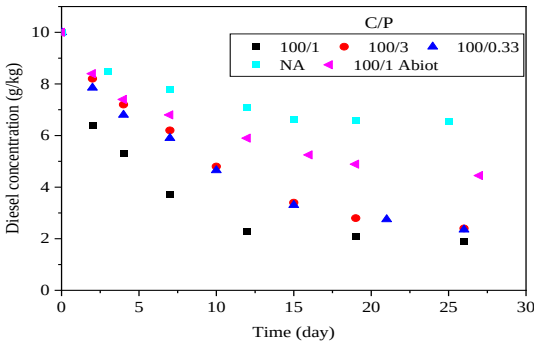

Fig. 7: Evolution of diesel concentration for different molar ratios
C/P in biotic mode. C/N = 100/10. Airflow rate = 1 L/min

the boundary layer and the characteristic time are very low, which means that the evaporation process remains very close to equilibrium. In such a case, the air is loaded with volatile compounds at equilibrium.

Effect of nutrient contents on diesel oil degradation

Mineral solutions of NH_4NO_3 and K_3PO_4 have been used as nitrogen and phosphorus sources under aerated conditions to amend the soil samples at different molar ratios of C/N/P: 100/10/1, 100/5/1, 100/25/1, 10/10/3, and 100/10/0.33 under an airflow rate of 1 L/min. Figs. 6 and 7 show the evolution of the diesel oil concentration for different molar ratios of C/N/P. The natural attenuation and the abiotic mode have been given in the same Figure. for comparison. It can be seen that the diesel oil concentration decreases with time under the investigated ratios. However, the diesel oil degradation decreases slowly in the natural attenuation; this means that the addition of an inorganic supplement of nitrogen (N) and phosphorus (P), and oxygen has a significant effect on the C/N/P ratio of the soil, which in turn has an impact on the diesel oil degradation in the soil.

The addition of specific nutrients, particularly N and P at low ratios, clearly shows that indigenous bacteria are able to metabolize diesel oil under low nutrient conditions. The constant k of diesel oil removal obtained after 26 days of treatment is more significant for the molar ratio C/N/P = 100/10/1 (0.136 (/day)), followed by the ratios of 100/5/1 (0.123 (/day)), 100/25/1 (0.115 (/day)), 100/10/0.33 (0.096 (/day)) and 100/10/3 (0.082 (/day)). In such a case, the diesel oil removal reaches 80, 75, 75, 76, and 76%, respectively. This is in agreement with the results previously found by some researchers, who reported that the ratio of 100/10/1 is often recommended for bioremediation of organic compounds [28-51]. It offers good diesel oil metabolization by indigenous bacteria. The addition of nutrients during the bioremediation is required to stimulate bacteria growth and, in this way, pollution removal [52]. The use of poultry manures and NPK fertilizer as bio-stimulating agents in a soil polluted with a petroleum hydrocarbon mixture increases the fuel removal by 73% [9]. The ddition of nutrients and oxygen to the contaminated soil can expedite the biodegradation process and increase the rate of biodegradation [53].

Nutrient amendments have a positive effect on the bioremediation of soil contaminated with hydrocarbons; however, when they are added in excess during the biodegradation of fuels, an inhibitory effect is revealed. In this study, the molar ratio C/N/P = 100/10/1 provides the best removal rate, while lower nitrogen and phosphorus contents reduce bacterial degrading power but increase

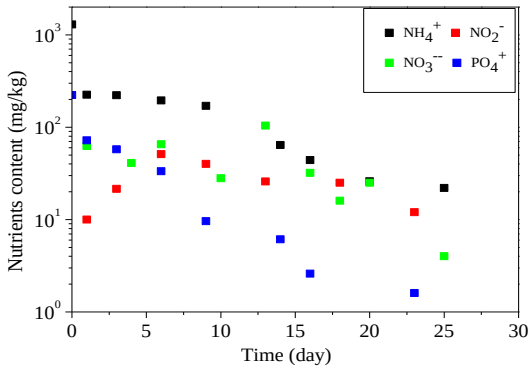

Fig. 8: Evolution of the nutrient contents in the soil C/N/P = 100/10/1, airflow rate =1 L/min

inhibition. The supply of nutrients and oxygen are still the key factors influencing the biodegradation process of diesel oil by indigenous bacteria.

Nutrient behaviour in the soil

The nutrient content evolution for the molar ratio C/N/P = 100/10/1 is illustrated in figure 8. It can be seen that the curves of nutrient content progress in a similar way except for nitrates and nitrites. The ammonium and phosphorous contents in the soil decrease from 1295 to 225 mg/kg and from 223 to 72 mg/kg, respectively, during the 1st day of treatment. This may be due to the potential assimilation of the phosphorus and ammonium by the indigenous soil bacteria to degrade the hydrocarbon compounds and also to the fixation of the cations by the soil particles [54]. Due to the short duration of the experiments, other mechanisms such as volatilization and nitrification, as well as interlayer cation fixation, existed in soil environments [55]. It can also be seen that the ammonium content is about 140 mg/kg after 10 days of treatment and decreases to 25 mg/kg on the 20th day. During the biodegradation process, NH_4^+ can be oxidized into NO_2^- and NO_3^- by enzymatic catalysis. At the same time, the phosphorus content is 9 mg/kg and decreases to 1 mg/kg. The nitrogen and phosphorus nutrients are well assimilated by microorganisms. Note that NO_2^- and NO_3^- concentrations were null initially and then increased until stability around a mean value that varies between 50 and 60 mg/kg, due to the soil pH [55].

CONCLUSIONS

Biodegradation by indigenous microorganisms is a promising technique to remediate hydrocarbon-impacted sites due to its eco-friendly feature. The choice of an adequate bioremediation method that reduces hydrocarbons to harmless compounds for the environment is essential for a successful bioremediation treatment technique.

The method used in this research allowed reducing the diesel oil concentration from 10 to 0.34 g/kg of sand using an airflow of 2 L/min and a C/N/P ratio of 100/10/1, which corresponds to a removal rate of 97% after 25 days. Nevertheless, since most of the diesel oil eliminated (about 87%) is the result of the evaporation and entrainment phenomena, the contribution of the bacterial activity is low; the number of bacteria in this case is about 10^8 CFU/g. However, with an airflow rate of 1 L/min, we obtained a removal rate of 80% with a biodegradation rate of 36%. This airflow rate gives the best biodegradation rate; the bacterial population in this case achieved 5.40×10^{10} CFU/g, which is the highest value reached. An air flow rate of 1 L/min and appropriate nutrients provide the best conditions to support indigenous micro-organisms' growth and sustain a better bioremediation of the polluted soil by a bio-stimulation approach, using nitrogen and phosphorous as nutrients sources.

Therefore, to improve the bacterial degradation of the organic pollutant, it is important to use an optimal airflow with a nutrient supply consisting of a nitrogen and phosphorus source in a molar ratio of 100/10/1. These conditions will result in high bacterial growth and thus increase the rate of degradation generated by the bacterial activity. This will reduce the quantity of gaseous oil entrained in the airflow rate.

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Authors wish to pay tribute to the memory of Professor BENTAHAR Fatiha who died on October 26, 2019.

Nomenclature

C	Inorganic carbon content (mg/g)
C_0	Initial diesel concentration (g/kg);
C_{biot}	Diesel concentration over time in biotic mode (g/kg);
C_{abiot}	Diesel concentration over time in abiotic mode (g/kg).

CO	Organic carbon content (mg/g)
d_h	Harmonic diameter (µm)
NA	Natural attenuation
ρ_s	Density of sandy soil particles (kg/m^3)
μ_{max}	Bacterial growth rate (/day)
T_R,	Total removal ratio
T_{bio}	Degradation ratio
T_{abiot}	Removal rate due to transfer and evaporation

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