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Biodegradation Potential of Crude Oil by *Pseudomonas aeruginosa* Strains and Analysis of Residual Oil by GC-MS

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Abstract

The potential biodegradation of crude oil was assessed by 50 strains of *Pseudomonas aeruginosa* cultured in a basal mineral medium using crude oil as a sole carbon source. The strains are isolated from both environmental and hospital samples (non-contaminated crude oil sites). After 28 days of incubation, more than 60% of crude oil was degraded and further converted into accumulated cell biomass. Therefore, the use of the bacterial consortium increases the percentage of biodegradation up to 67 %. The analysis of residual crude oil by Gas chromatography-mass spectrometry (GC-MS) confirms the results that show that *Pseudomonas aeruginosa* could be effective in the biodegradation of crude oil.

Keywords: Crude oil; Biodegradation; *Pseudomonas aeruginosa*; GC-MS

Introduction

Petroleum hydrocarbons are important energy resources, which are used not only by industry, but also in our daily life. Petroleum is a major pollutant of the environment as well. Due to its complicated composition, petroleum has the potential to elicit multiple types of toxic effects. It can cause acute lethal toxicity, sub-lethal chronic toxicity or both depending on the exposure, dosage, and the organism being exposed [1]. On the one hand, Prolonged exposure and high oil concentration may cause the development of liver or kidney disease, it is also probable to damage the bone marrow and to increase the risk of cancer [2,3].

On the other hand, some components of petroleum have the potential to bioaccumulate within susceptible aquatic organisms and can be passed by trophic transfer to other levels of the food chain [4,5]. The presence of microorganisms with the appropriate metabolic capabilities is the most important requirement for oil spill bioremediation [6].

The traditional treatment of oily wastewater, such as containment and collection using floating booms, adsorption by natural or synthetic materials, etc., cannot degrade the crude oil thoroughly. However, the microbial bioremediation, as a natural elimination of these pollutants; have long been considered as one of the predominant hydrocarbon degrading agents found in the environment, which are free living and ubiquitous [1].

Accordingly, our study aims at evaluating the biodegradation of crude oil by Pseudomonas aeruginosa; isolated from non-contaminated sites in pure culture and in a mixed bacterial consortium ex-situ.

Materials and Methods

Biodegradation Assays

Crude oil degrading bacteria were carried out under aerobic condition in the mineral salt media (MSM) with

crude oil (Arabian Light) as the sole source of carbon, the MSM with the following composition (g/L): KH_2PO_4: 0.68; $MgSO_4.7H_2O$: 0.35; Na_2HPO_4:1.7; $CaCl_2$:0.02; NH_4NO_3: 1; $FeSO_4$: 0.004,supplemented with a solution of trace elements (0, 01% final concentration) containing in g/L: $CuSO_4$: 0.05; H_3BO_4:0.1;$MnSO_4$: 0.1;$ZnSO_4$: 0.1;Na_2MoO_4: 0.1;$CoCl_2$:0.1. The pH was adjusted to 7. The media was amended with 0,5 % filter sterilized crude oil (v/v).

The individual and the mixed bacterial consortiums from overnight culture at the log phase of growth were transferred to 250 mL conical flasks; each containing 100 mL of sterile mineral salts medium with (0.5% v/v) of crude oil. The experiment was carried out in triplex and non- inoculated flasks constituted the controls. All flasks were incubated on a shaker (Lab-line, Environ shaker - USA) at 150 rmp at 30°C for determined intervals of time (14,21, and 28 days).

Extraction and Analysis of Residual Oil

The Residual concentrations of crude oil from cultures and controls were determined by liquid–liquid extraction, which is carried out by a double volume of chloroform (twice 70 ml) using separating funnels; the extract was treated with 2g of anhydrous sodium sulphate to remove the moisture, thus, the chloroform containing the residual hydrocarbons was decanted and air dried. After chloroform evaporation, the residual oil was quantifed. The resulting residual oil is taken to determine the percentage biodegradation by applying the equation described below by Fusey and Oudot [7]:

$$\textit{Biodegradation Percentage} = \frac{(pi - pev) - pr}{pi - pev} \times 100$$

pi: *Quantity of initial crude oil.*

pev: *Quantity of crude oil evaporated.*

pr: *Quantity of residual crude oil.*

Gas Chromatography Analysis

After its being extracted at the end of the 28 days of incubation, the residual crude oil was quantified chromatographically via the gas chromatography coupled to mass spectroscopy (GC-MS).

The extracted oil was detected spectrophotometrically at the National Scientific Research Center of Rabat (Morocco). The Gas Chromatography/Mass Spectrometry (GC/MS) analysis were performed by a Hewlett-Packard gas chromatographer (HP 6890) coupled with a mass spectrometer (HP 5973). The fragmentation was performed by electron impact at 70 eV. The column used was HP-5MS (30 m x 0.25 mm, film thickness: 0.25 μm). The carrier gas is helium whose flow is fixed at 1.5 ml. min^{-1}. The injection mode was split (split ratio: 1/70, flow rate112 ml min^{-1}). The column temperature was programmed from 50 to 200°C at a heating rate of 4°C. min^{-1}, during 5 min. For the chromatographic analysis, the residual oil was diluted in methanol (1/20 v/v).

Results and Discussion

After each incubation period (14, 21 and 28 days), we estimated the crude oil degradation by isolated *P. aeruginosa* strains according to the equational ready described above. The results are in the form of a histogram. Were sorted to a histogram showing the biodegradation of crude oil by environmental strains and another one for hospital strains (Figures 1 & 2).

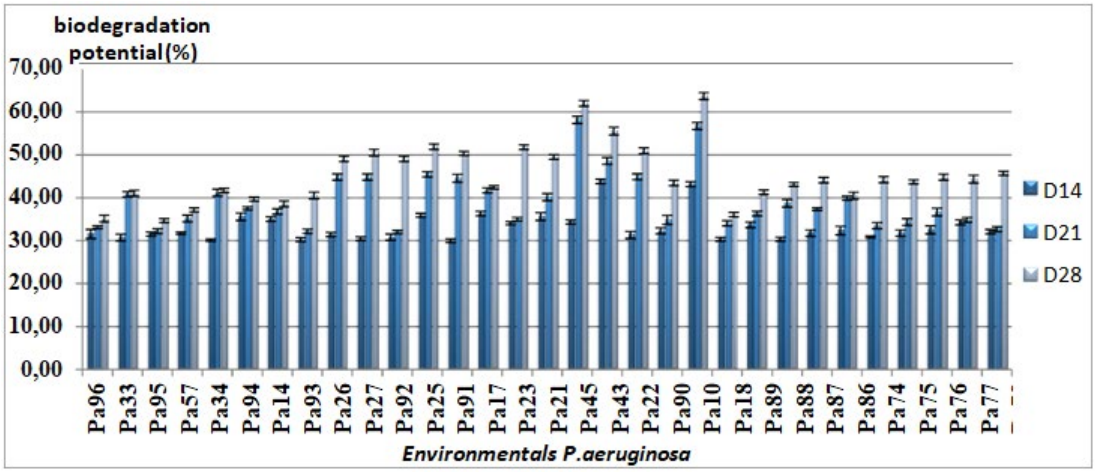

Figure 1: Biodegradation Potential of crude oil by environmentals *P. aeruginosa* strains.

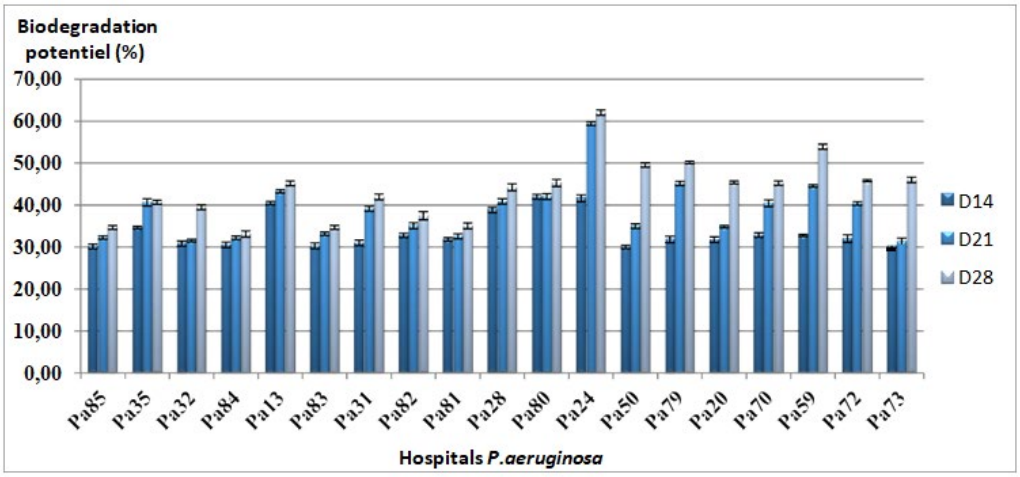

Figure 2: Biodegradation Potentiel of crude oil by hospitals *P. aeruginosa* strains.

Initially, crude oil appeared as either a large stretch covering above the aqueous medium or attached on glass wall. Later on, the large stretch or attachment of petroleum turned gradually into dispersed oil drops at a medium [8].

According to the results, we note that among the 50 *P. aeruginosa* strains tested, 11 strains possess a crude oil biodegradation potential located in the vicinity of 50% and 60%, 27 strains have a potential of between 40% and 50% and 12 strains have a biodegradation potential of between 30% and 40% (Figures N ° 1 and N ° 2). In another way, most of the strains tested have a percentage of biodegradation between 40% and 50% and only 3 strains show a potential slightly exceeding the value of 60%.

The minimum and the maximum values recorded for the biodegradation potentials or percentage are summarized in Table 1.

Biodegradation potential (%)/ Days		D14	D21	D28
Environmentals strains	Minimum values	30,01 (Pa 91)	32,04 (Pa 92)	34,69 (Pa 95)
	Maximum values	43,83 (Pa 43)	58,16 (Pa 45)	63,71(Pa 10)
Hospitals strains	Minimum values	30,02 (Pa 50)	31,37 (Pa 73)	33,11(Pa 84)
	Maximum values	42,19 (Pa 80)	59,42 (Pa 24)	62,05 (Pa 24)

Table 1: Minimum and maximum values of the biodegradation potentials of crude oil of 50 *P. aeruginosa* strains tested.

From the results in Table N°1, we note that the minimum values for the biodegradation potential of crude oil are around 30% for an incubation period of 14 days. However, for the same incubation period (14 days), the maximum values exceeding 42% (Figure 2); This reflects the difference in the crude oil biodegradation kinetics between strains.

In addition, the maximum values obtained for the biodegradation potential are very heterogeneous, ranging from 42% to approximately 63%, reflecting their varying ability to use some components of crude oil.

The values obtained for the biodegradation potentials are relatively high compared to those reported by the bibliographic data; in this regard, a comparative study of the biodegradation of crude oil carried out by Kheira Hammadi, et al. [8], using *Pseudomonas aeruginosa*, *Aspergillus terreus* and Candida sp. petroleum showed that only *Pseudomonas aeruginosa* strains have a high percentage of biodegradation. In addition, Zhang [10] found that after 8 days of incubation, 58% to over 60% of the initial concentration of crude oil (0.7 g / L) were consumed.

Biodegradation of crude oil microbially causes decomposition or oil components of transformation to other organic compounds [11].In other words, crude oil biodegradation can be described as the conversion of chemical compounds by microorganisms in energy, biomass and organic products. Due to the good utilization of hydrocarbons, *Pseudomonas aeruginosa* is especially investigated for biodegradation of crude oil [10].

The mixed cultures carried out as well as the results of biodegradation obtained are grouped together in Table2.

Strains	Biodegradation potentiel (%)
Pa10+Pa45	66,87
Pa10+Pa45+Pa24	67,05
Pa10	63,71
Pa45	62
Pa24	62,05

Table 2: Biodegradation potentials (%) of crude oil mixed cultures

Legend: Pa10 and Pa 45 are environmental strains; Pa 24: Hospital strain.

According to the results, we notice that the biodegradation potential of crude oil increased in the presence of a mixed culture compared to a pure culture, suggesting that these strains metabolize various substrates. The mixed cultures carried out consist of strains of *P. aeruginosa* having shown a high percentage of biodegradation (more than 60%) after 28 days of incubation.

In this regard, it is reported by some authors who have shown that the biodegradation of oil is improved in the presence of a consortium of bacterial species compared to a single species [12,13,10]. Cocultivation of *Pseudomonas aeruginosa* with a microbial consortium could also enhance bioavailability and hence the biodegradation of crude oil due to its ability to produce rhamnolipids [14].

The crude oil contains thousands of individual hydrocarbons and related compounds. Their main components are saturated (n- and branched-chain alkanes and cycloparafins rings) and aromatic and polynuclear compounds (PAHs) and resines and asphaltenes (heterocyclics, oxygenated hydrocarbons), Westlake [15] reports that no single microbial species have the enzymatic ability to metabolize more than two or three classes of compounds commonly found in crude oil. A consortium composed of many different bacterial species is therefore needed to degrade the crude oil significantly.

Indeed, the use of a bacterial consortium provides some advantages in the case where the toxicity of the pollutants is important or when there is a lack of appropriate microorganisms (quantitatively and qualitatively) [13].

The biodegradation of crude oil was further confirmed by detecting the compositions of residual crude oil using gas chromatography-mass spectrometry (GC-MS) (Figures 3-5). The first Profile (Figure 3) as a control presents the compositions of crude oil in the non-inoculated flask after 28 days of biodegradation while; the second and the third profiles (Figures 4 & 5) show the compositions of the crude oil after 28 days of incubation in the inoculated flasks. It was found that most peaks in the first profile disappeared in the second and the third profiles, which indicated an apparently high rate of utilization of crude oil by *Pseudomonas aeruginosa.*

According to gas chromatographic profiles, *Pseudomonas aeruginosa* Pa10 and the consortium (Pa10 + Pa45) degraded most oil fractions, with a retention time avoid 50 min after 28 days of incubation in a crude oil-containing medium, we noticed a slight decrease in the peaks regarding the consortium profiles, this corresponded well with the literature. Thus, the results of biodegradation potential obtained were corroborated by GC-MS analysis. In another work, the GC-MS result shows that the relative abundance of the peak reduced considerably for isolated bacterial organisms depending on potential of oil degradation compared to control. In general, the results showed that many compounds were completely degraded or transformed into less complicated compounds.

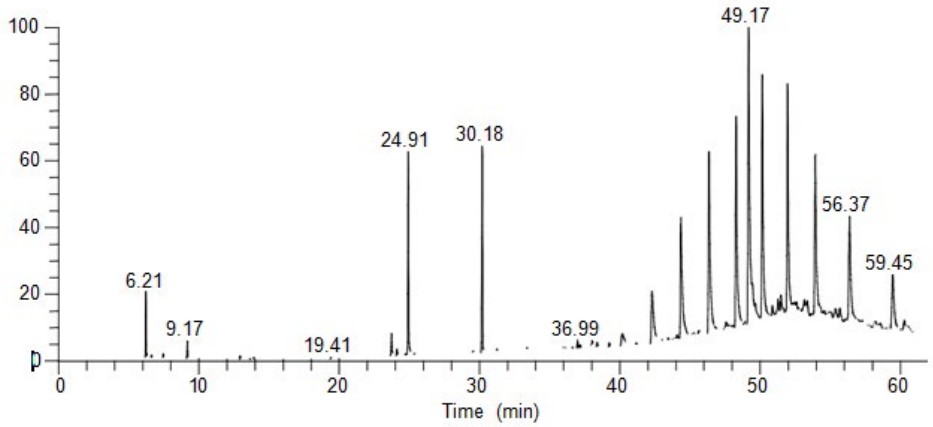

Figure 3: Gas chromatographic-mass spectrometry analysis of untreated crude oil after 28 days of incubation.

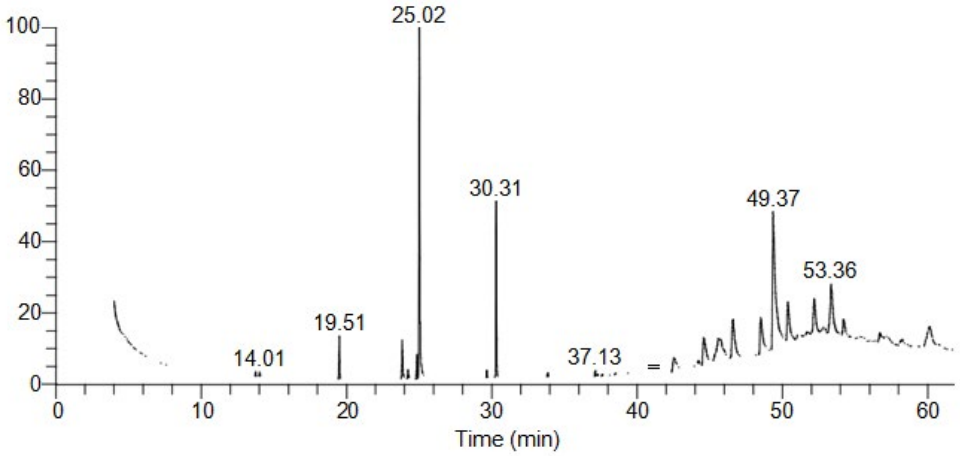

Figure 4: Gas chromatographic-mass spectrometry analysis of residual crudeoil after 28 days of incubation with *Pseudomonas aeruginosa* Pa 10.

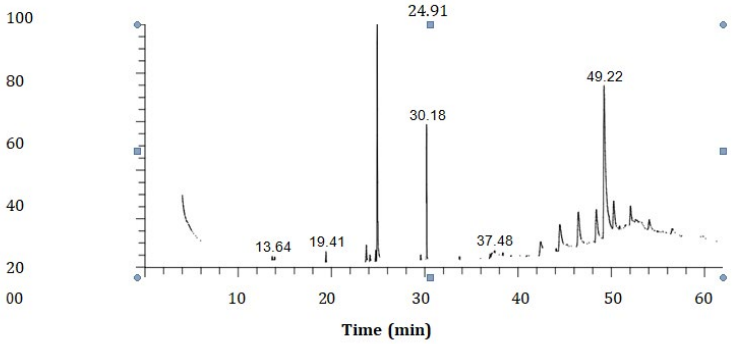

Figure 5: Gas Chromatographic-Mass Spectrometry Analysis of Residual Crude Oil after 28 Days of Incubation with *Pseudomonas aeruginosa* Pa10+Pa45.

Conclusion

The individual bacterial strains have biodegradation abilities less than their combination (consortium) because the hydrocarbon mixtures differ markedly in volatility, solubility, and susceptibility to degradation and the necessary enzymes needed for biodegradation cannot be found in a single organism. The mixed bacterial culture could carry out a maximum degradation (67,05%) for the studied crude oil at 30°C after 28 days of incubation. The results of this study suggest that the *Pseudomonas aeruginosa* isolated strains may have the potential for bioremediation of crude oil polluted sites indicating the possibility of their use either singly or in consortium.

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