

Potential of Selected Microbial Strains to Degrade the Gasoil of Hydrocarbon Polluted Soil

Ali Zazoua, Anis Zazoua, Ahcen Taleb, Nicole Jaffrezic-Renault

Abstract—Although oil-based drilling fluids are of paramount practical and economical interest, they represent a serious source of pollution, once released into the environment as drill cuttings. The aim of this study is to assess the capability of isolated microorganisms to degrade gasoil fuel. The commonly used physicochemical and biodegradation remediation techniques of petroleum contaminated soil were both investigated. The study revealed that natural biodegradation is favorable. Even though, the presence of heavy metals, the moisture level of (8.55%) and nutrient deficiencies put severe constraints on microorganisms' survival ranges inhibiting the biodegradation process. The selected strains were able to degrade the diesel fuel at significantly high rates (around 98%).

Keywords—Biodegradation, Gasoil, Pollution, Microbial strains, Hydrocarbon, soil pollution

I. INTRODUCTION

THE accumulation of all kinds of releases into the environment has become a major concern for many years, in particular since many of these are highly toxic. This has triggered a strong awareness worldwide and considerable research activity to cope with the problem [1-3]. The challenge might seem to be lost in advance, given the tremendous increase in industrial activity, consumption and development of intensive agriculture. Furthermore, one of the most aggressive aspects of global pollution by synthetic compound is driven by the growth of hydrocarbons release into the environment. Indeed, the oil industry generates a series of solid and liquid wastes during drilling operations and cleanup of industrial facilities that contain heavy crude oil compounds; alkanes, paraffins and aromatics in particular [4,5]. Many hydrocarbon compounds exhibit hydrophobic chemical structure highly resistant to natural biodegradation, persisting for years or even decades. Moreover, many of these compounds are toxic, presenting therefore serious implications on ecosystems and human health risks [6,7]. These health risks are mainly related to the propagation of hazardous molecules into the environment, and their transmission into aquifers and food chains. The soil cleanup processes in use are varied and new technologies are being developed over the years. Nevertheless, the most commonly used are the thermal treatment and physicochemical approaches, in addition to biological methods, which take advantage of the decontamination capability of microorganisms, often seen as restrained to a specific class of compounds such as petroleum hydrocarbons.

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Bioremediation is a well established technique and is by far the most attractive decontaminating approach [8]. Bioremediation possess many valuable features, including low implementation cost, high efficiency rate and more importantly the ability to convert contaminants to harmless end products. In fact bioremediation is based on the complete mineralization of petroleum products, which do not generate any toxic compound, unlike physicochemical processes that often involve a transfer of pollution from one site to another [9].

The purpose of this study is to assess the microbial capability to degrade hydrocarbons from a quagmire contaminated by drilling fluids. In this respect, we used the bioprocess "Landforming" to reduce hydrocarbon content in the quagmire. This is facilitated by the fact that bacteria use the hydrocarbon chains as a carbon source, essential for their growth, resulting in a total breakdown of hydrocarbons that will be used to determine the removal efficiency of organic pollutants by biological treatment. The inoculums were prepared from strains *Pseudomonas aeruginosa*, *Klebsiella oxytoca* and *Staphylococcus aureus*.

II. MATERIALS AND METHODS

Samples were taken from a portion of the oil field "Ourhoud", located in the basin BERKINE to 260 km south-east of Hassi Messaoud and 1200 km south-east of Algiers (Algeria). Weather data recorded at this site indicates that the average temperature is below 45 ° C in summer, with an absolute maximum temperature of 60 ° C. The samples were extracted from a contaminated soil at 20-50 cm depth, at various locations. The samples were collected in plastic bags and placed in a cooler. These samples were subsequently air dried for 48 hours and then filtered through a 2 mm sieve to remove large solid fragments, and subjected to grinding in order to reduce the particle size and enhance contamination homogeneity.

A. Sample preparation

After vigorous shaking to further improve homogeneity, the sample was weighed. To extract Friuli, we added to the sample, 50g of anhydrous sodium sulfate and 50ml of carbon tetrachloride in a pot, placed in a bath of ice water to prevent loss through heating. We mixed the content in a blender for 15 minutes. After the separation phase, we collected the carbon tetrachloride in a beaker containing 1g of anhydrous sodium sulfate. Carbon tetrachloride was poured into a second beaker and then transferred to a volumetric flask.

B. Experiment description

Landforming biological treatment is based on the biodegradation of total hydrocarbons. The experiments were performed in a wooden structure. Portions of soil were divided into three containers as follows:

- A control set containing the polluted soil and indigenous flora.
- A biostimulation set containing the polluted soil and indigenous flora with nutritional support to assess the potential of increasing the rate of biodegradation by adding nutrients (N, P).
- A Bioaugmentation set, containing soil, nutrient medium and inoculums of the mixed culture (the three bacterial species) to evaluate their implication on biodegradation speed.

C. Preparation of inoculums

The bacteria used in this study were provided by the Microbiologic Laboratory of Jijel University.

- *Chemical composition of culture medium*

The culture medium composition was: 5g of K_2HPO_4 , 2g KH_2PO_4 , 2g $MgSO_4$, 0.1g NaCl, 3g $NaNO_3$ and 5g yeast extract all mixed in 1L of distilled water. The pH was adjusted to 7.5 using HCl solution. The resulting medium was then autoclaved at 120°C, during 20 minutes. 2% of diesel was added to the culture media afterwards.

- *Culture condition*

The inoculum was prepared from strains *Pseudomonas aeruginosa*, *Klebsiella oxytoca* *Staphylococcus aureus*. Colonies of these strains aged 24 h were seeded into 200ml of culture medium, then inoculated into a vial. The solution was placed on a shaker to obtain an anaerobic culture at a temperature of 30 °C for 24 h. The appearance of disorder in the medium was a good indicator of bacterial growth. A volume of 0.1ml of culture was deposited into an Erlenmeyer flask containing 200 ml of culture medium. Again, the preparation was placed in an incubator at 60tr/min stirring for 24 hours. The prepared culture was centrifuged at 3000rpm. The pellet obtained was washed twice and then diluted in sterile saline solution. The prepared inoculum was brought in by spraying.

D. Bioremediation monitoring

The containers were kept at room temperature; aeration was performed regularly throughout the examination duration. Samples were taken out for assessment weekly, and gas chromatography (GC/MS Shimadzu, apolar chromatographic column SE 30) and FTIR (Schimadzu Irtfinity-1) were used to monitor the biodegradation level. The determination of heavy metals was carried out by atomic absorption spectroscopy.

TABLE I
QUANTITATIVE AND QUALITATIVE ANALYSIS OF HYDROCARBONS

Study	parameters	levels
Quantitative qualitative	Totals hydrocarbons	160 g/kg
	Saturated hydrocarbons	20.57%
	Aromatic hydrocarbons	3.06%
	Resins	71.16%
	Asphaltenes	5.20%

TABLE II
MEASURED HEAVY METALS CONTENT AND PARAMETERS INDICATORS OF POLLUTION. (*) NETHERLANDS, CIRCULAR ON TARGET
VALUES AND INTERVENTION VALUES FOR SOIL REMEDIATION, 2004

	Cadmium (ppm)	Chromium (ppm)	Lead (ppm)	Manganese (ppm)	Copper (ppm)	Zinc (ppm)	Iron (ppm)	Nickel (ppm)	PO_4^{3-} (Mg/L)	NO_3^- (Mg/L)	NO_2^- (Mg/L)	DBO_5 (Mg/L)	DCO (Mg/L)	Dissolved Oxygen (Mg/L)
Standards (ppm)*	0.8	81	19	850	34	120	2000	100	-	-	-	-	-	-
Levels	3.84	42.04	105.125	34.655	8.165	141.1	3519	24.94	0.8	0.5	3.44	20	260	7.8

III. RESULTS AND DISCUSSION

Table I shows the results of quantitative and qualitative study of total hydrocarbons. The oil content was 160 g/kg, which represents a percentage of 16%. It is worth noting that the current standard for a healthy soil is 2%. This means that we are in the presence of a polluted soil. The test results shown in Table 1 reveal that the resins are the predominant component of total hydrocarbons constituents with 71.16% content. The resins are more resistant to natural biodegradation [10]. The physicochemical analysis

of soil showed a temperature equal to 29 °C. This value allows the normal growth of bacteria. This parameter can affect the biodegradation of oil by altering the physiological activity of microorganisms and consequently the speed and the degree of the degradation [11]. The moisture level of the soil obtained was 9.52%. This is relatively low but still sufficient for a quagmire. However, it should be noted that low humidity may have a negative effect on biodegradation. A supply of water allows enhancement of bacterial process and microorganisms/pollutant contact [12]. Soil pH is 8.55.

It is an alkaline pH, thereby promoting the growth of bacteria and increasing hydrocarbons biodegradation efficiency.

A. Granulometric analysis of soil

Granulometric analysis allowed identification of soil texture. This analysis shows that the soil is sandy in nature (Argil ($< 2\mu\text{m}$) 0.02%, Silt (2 - 62 μm) 2.43% and Sand (62 - 2000 μm) 97.55 %), which promotes the transport of nutrients and oxygen to the indigenous flora in the medium, responsible for the biodegradation [13]. Table. 2 shows the measured heavy metals content. The atomic spectrometry has revealed the existence of heavy metals: Cd, Cr, Pb, Mg, Cu, Ni, Zn and Fe. We noticed a rather high rate of some metals that go far beyond the standard values, for instance cadmium, lead and iron. In the presence of these metals, biodegradation is more difficult. Therefore, selected microorganisms behavior should be insensitive to heavy metals [14].

B. Determination of parameters indicative of pollution

The results in Table II indicate a deficiency of the elements nitrogen and phosphorus. Soil pollution by hydrocarbons (gasoil) results thus in a shortage of nitrogen and phosphorus, which limit the biodegradation efficiency of hydrocarbons. Enrichment of soil with these fertilizers is therefore necessary. Conversely, the concentration of dissolved oxygen obtained was sufficient for a natural biodegradation process to take place. The DBO5/DCO ratio measured was 0.077. The study shows that the diesel is poorly biodegradable (7.7%) under natural conditions compared to Arabian Light crude oil (exceeding 65%), while fuel oil was 11% reported by Oudot (1984) [15] and Oudot (2000) [16].

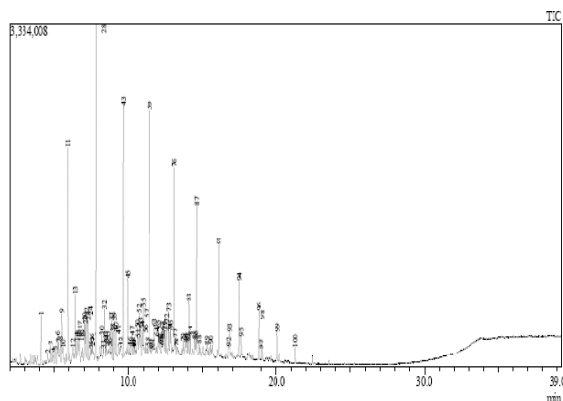


Fig. 1 Chromatogram of control set containing the polluted soil and indigenous flora (apolar chromatographic column SE 30)

The contamination level of fertilized soil at the fourth week was still high, because of the limitation of the biostimulation effect provided by soil indigenous microorganisms (fig. (b) 3). Conversely, the paralleled bioaugmentation degradation was mostly completed (fig. (b) 4). This is explained by the disappearance of the peaks and the appearance of hydrocarbons residues (1-2% of initial amount). The disappearance of these peaks may be attributed to either evaporation or biodegradation. The possibility of evaporation is obviously excluded since the diesel evaporates at 180 ° C, while the containers were kept at about 28 ° C. Therefore, biodegradation phenomenon should have taken place.

This is probably due to the nature of diesel itself, which is composed of several types of hydrocarbon constituents of different classes more difficult to assimilate by microorganisms, hence the need for biological treatment.

C. Evolution of hydrocarbons biodegradation

Chromatographic analysis of the quagmire (treated) provided the results presented in Figures 1 to 4. These results were obtained by Gas chromatography-mass spectrometry (GC / MS) of the quagmire samples and crude gasoil.

The comparison between the resulting spectrum and chromatogram of gasoil sample (fig. 1. and fig. 2.) reveals a great similarity (hydrocarbons from C8 to C20). The most prominent peaks correspond to n-alkanes. We noticed the absence of lighter fractions in the sample, which may be explained by a possible volatilization. These results show that the major contaminant of the quagmire is merely gasoil.

The biostimulation chromatogram (fig. (a) 3) reveals the similarity with the control. This absence of concentration changes of hydrocarbons in the soil, means that no biodegradation occurred. However, the figure representing the chromatogram of the soil subjected to bioaugmentation experience (fig. (a) 4) reveals that significant biodegradation occurred during the first two weeks.

This is in agreement with the findings reported by DeMello *et al* [17], suggesting that the biodegradability rate of the treated site should exceed 50% within the first five days of testing. The n-alkanes are degraded more rapidly than iso-alkanes, which in turn are more rapidly degraded than aromatic compounds, which remain the most readily degraded compounds followed by naphthenes. This is consistent with the outputs validated by Prince *et al* [18].

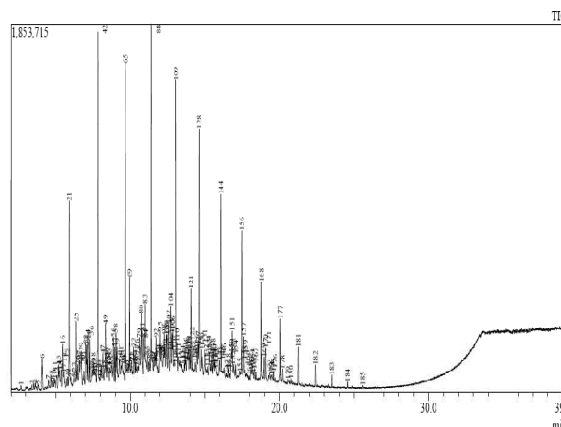


Fig. 2 Chromatogram of crude gasoil (apolar chromatographic column SE 30)

This is further confirmed by the presence of n-alkane and iso-alkane residues in the treatment site. Additionally, it is noteworthy to mention that these residues are more importantly present in the biostimulation case. These altogether prove the presence of the biodegradation process.

The major IR absorption region of hydrocarbons according to Bocard [19] is located between 3125 cm^{-1} and 2800 cm^{-1} part of IR spectrum, due to carbon-hydrogen bonds. Therefore, our analysis was focused on this region, which includes the bands characteristic of the major functional groups, and are used to monitor and confirm the presence or absence of hydrocarbons. Fig. 5 show the IR spectra of the three samples mentioned above.

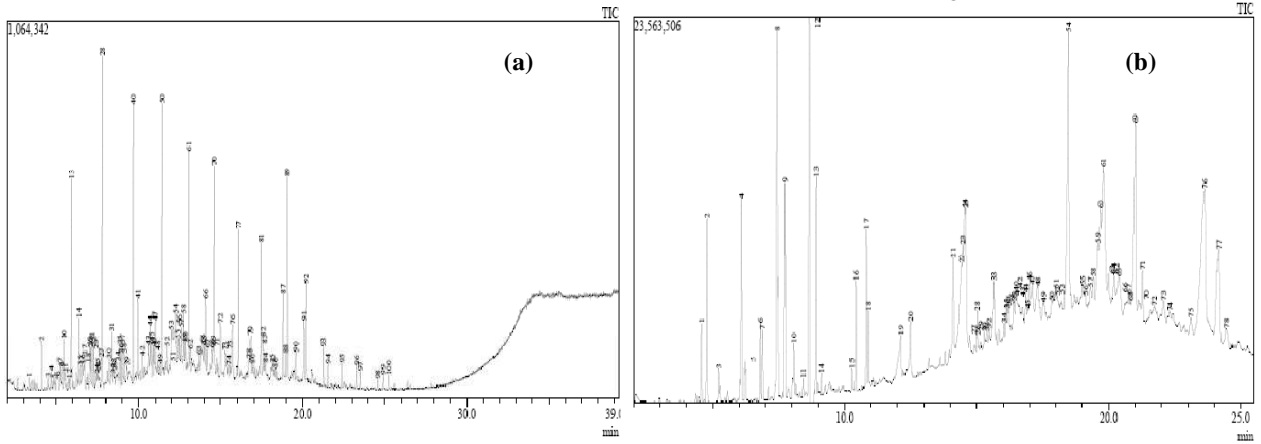


Fig. 3 Chromatogram of biostimulation set containing the polluted soil and indigenous flora with nutritional support to assess the potential of increasing the rate of biodegradation by adding nutrients (N, P) (a) after 2 weeks incubation, (b) after 4 weeks incubation

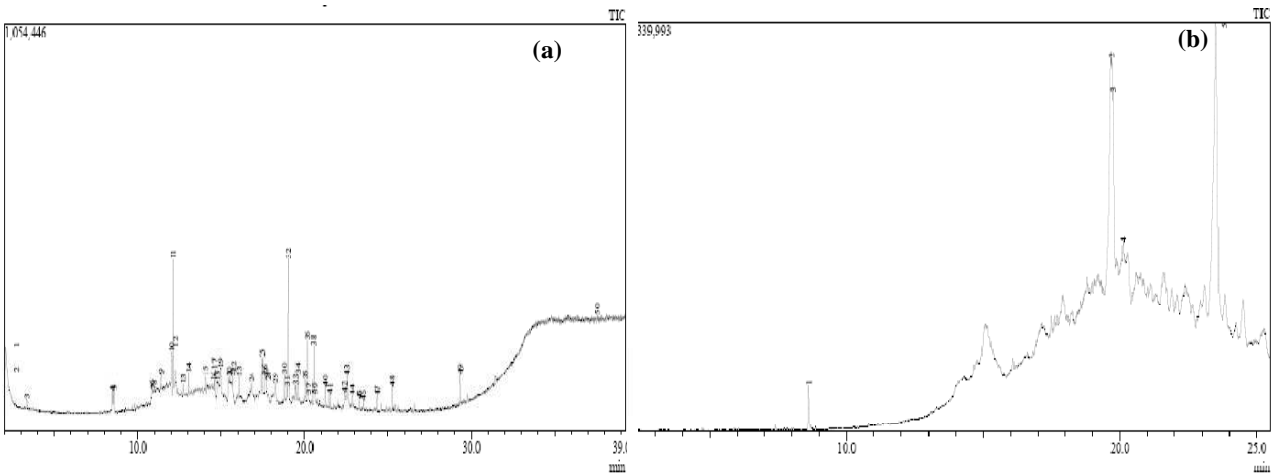


Fig. 4 Chromatogram of bioaugmentation set, containing soil, nutrient medium and inoculums of the mixed culture (the three bacterial species) to evaluate their implication on biodegradation speed (a) after 2 weeks incubation, (b) after 4 weeks incubation

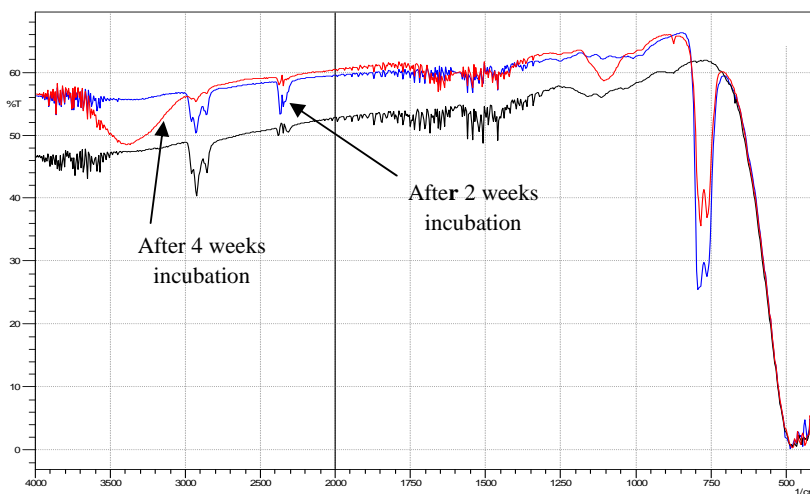


Fig. 5 FT-IR characterization of bioaugmentation set, containing soil, nutrient medium and inoculums of the mixed culture (the three bacterial species) to evaluate their implication on biodegradation speed

The results showed a steady decrease in bands intensity located at 3000 - 2854 cm^{-1} , 2367 cm^{-1} and 1458 - 1379 cm^{-1} corresponding to bioaugmentation (fig. 5), until their total disappearance in the last week. Compiled from the literature, these bands are attributed to C-H stretching vibrations and bending vibrations of alkanes, alkenes and aromatics. The above results are indicative of biodegradation of several fractions, constituting the studied hydrocarbon. These results are in perfect agreement with what was found by chromatography.

IV. CONCLUSION

The outcome of this study shows that the diesel is the major pollutant of the quagmire, with a fairly high rate. The physicochemical study revealed that the natural biodegradation is favorable. Nevertheless, the presence of heavy metals, moisture level and nutrient deficiency inhibit the biodegradation process. The obtained results showed that the investigated diesel consists of different classes of compound poorly assimilated by microorganisms, hence the need for a bio-treatment to enhance its degradation. A significant reduction in the concentration of hydrocarbons was observed. Indeed, the selected strains were able to degrade diesel efficiently with a high rate (around 98%).

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REFERENCES

- [1] O. Radwan, S.S., 2008. Microbiology of oil-contaminated desert soils and coastal areas in the Arabian Gulf. *Soil Biology* 13. In: Dion, P., Nautiyal, C.S. (Eds.), *Microbiology of Extreme Soils*. 13, 275-298
- [2] Mahmoud, H., Al-Hasan, R.H., Khanafer, M., Radwan, S.S., 2010. A microbiological study of the self-cleaning potential of oily Arabian Gulf coasts. *Environ. Sci. Pollut. Res.* 17, 383-391
- [3] Ijah, U. J. J., Ndana, M., 2003. Stimulated biodegradation of crude oil in soil amended with periwinkle shells. *The Environmentalist*. 23, 249-254
- [4] Iquatt, C.B., Oyewole, O.A., Abioye, O.P., 2006. Bioremediation of petroleum polluted soil. *International Journal of Natural and Applied Science*. 1, 21-25
- [5] Ismailov, N. M., 1985. Biodegradation of oil hydrocarbons in soil inoculated with yeasts. *Microbiology* 54, 670-675
- [6] Khan, N., Warith, M.A., Luk, G., 2007. A comparison of acute toxicity of biodiesel, biodiesel blends, and diesel on aquatic organisms. *J. Air Waste Manage.* 57, 286-296
- [7] Lapinskiene, A., Martinkus, P., Rebzdaite, V., 2006. Eco-toxicological studies of diesel and biodiesel fuels in aerated soil. *Environ. Pollut.* 142, 432-437
- [8] Bento, F.M., Camargo, F.A., Okeke, B.C., Frankenberger W.T., 2005. Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. *Bioresource Technol.* 96, 1049-1055
- [9] Prince, R., Parkerton, T.F., Lee, C., 2007. The primary aerobic biodegradation of gasoline hydrocarbons. *Environ. Sci. Technol.* 41, 3316-3321
- [10] Samanta, S.K., Singh, O.M., Jain, R.K., 2002. Polycyclic aromatic hydrocarbons: environmental pollution and bioremediation – review, *Trends in Biotechnology*. 20, 243-248
- [11] Larpent, M-C., Sanglier, J.J., 1992. *Biotechnologie- principes et méthodes*, Edition, Doin éditeurs, Paris, 704
- [12] Lecomte, P., 1998. *Les sites pollués : traitement des sols et des eaux souterraines*, 2ème édition, Lavoisier, Paris, 130
- [13] Eriksson, M., Ka, J.O., Mohn, W.W., 2001. Effects of Low Temperature and Freeze- Thaw Cycles on Hydrocarbon Biodegradation in Arctic Tundra Soil. *Applied and Environmental Microbiology*. 67, 5107-5112
- [14] Fredrickson, J.K., Hicks Compuestos, R.J., LI, S.W., Brockman, F.J., 1988. Plasmid incidence in bacteria from deep subsurface sediments. *Appl. Environ Microbiol.* 54, 2916-2923
- [15] Oudot, J., 1984. Rate of microbial degradation of petroleum components as determined by computerized capillary gas-chromatography and computerized mass-spectrometry. *Mar. Environ.* 13, 277-302
- [16] Oudot, J., 2000. Biodégradation du fuel de l'Erika. *Life Science*. 323, 945-950
- [17] DeMello, J.A., Carmichael, C.A., Peacock, E.E., Nelson, R.K., Arey, J.S., Reddy, C.M., 2007. Biodegradation and environmental behavior of biodiesel mixtures in the sea: an initial study. *Mar. Pollut. Bull.* 54, 894-904
- [18] Prince, R., Haitmanek, C., Lee, C.C., 2008. The primary aerobic biodegradation of biodiesel B20. *Chemosphere*, 71, 1446-1451
- [19] Bocard, C., 2006. *Marées noires et sols pollués par les hydrocarbures*, Edition Technip, 234-239.

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