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# On the relevance of biosurfactant for contaminated soil and diesel degradation

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**ABSTRACT**

Contaminated sites are areas where, due to accidental spills or illegal deposits, polluting substances are present in the soil and are dangerous for humans and the environment. These are hydrocarbon contaminants, in particular the compounds making diesel. We know many engineering techniques for remedying it; some of them have been developed in recent decades, called bioremediation. They exploit the degradation of contaminants by microorganisms, for a lower impact on the ecosystem and considerable economic savings. Microorganisms are suitable for diesel degradation, capable of degrading a good percentage of contaminant. In contaminated microcosms we have a much higher $CO_2$ production than biotic controls (uncontaminated microcosms), with a progressive mineralization of diesel, data confirmed by gas chromatograph analyzes. Reductions of order of 70% for diesel are also obtained. By in-depth analyzes on the gas chromatograph, it can be highlighted that low molecular weight compounds are degraded in percentages greater than 90%, while high molecular weight compounds are reduced by 35-40%. Microbial populations are therefore able to use pollutants as a source of carbon and energy. There is still a limited knowledge about processes and microorganisms involved in the degradation of hydrocarbons in marine environment. Then it is of primary importance to deepen the phylogenetic and functional diversity of microbial communities, virtually involved in the biodegradation of hydrocarbons. Highly contaminated sites are unexplored sources of microorganisms with high potential for studying bioremediation processes and for the growth of new strategies in the field of environmental remediation.

***Keywords*:** Biomaterials, Bioremediation, Soil degradation, Nanobiotechnology, Bioeconomy

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## 1. INTRODUCTION

Since the beginning of the twentieth century, oil, as a transportable and easily usable energy source, has been considered one of the most important raw materials in the world. It is a natural and heterogeneous mixture of hydrocarbons, mainly represented by alkanes with different chain length and different points of branches, cyclo-alkanes, mono-aromatic or polycyclic aromatic hydrocarbons.

Other components present in petroleum, in a lower percentage than hydrocarbons, are represented by resins and asphaltenes, which contain nitrogen, sulfur and oxygen, with possible presence of phosphorus and heavy metals (nickel and vanadium) in traces. The composition of the oil varies widely, depending on the type of crude oil, and each present component possesses various physico-chemical properties, as viscosity, solubility, absorption capacity, bioavailability, toxicity [1]. Although petroleum hydrocarbons are significant energy resources and one of the raw materials for different types of industries, they are classified as priority pollutants. Many constituents such as benzene, toluene, ethylbenzene and xylene (BTEX) are highly toxic, due to the presence of haemotoxic, carcinogenic and teratogenic components [2,3]. Alterations in metabolic reactions, in hormonal balance, acute mortality, necrosis, hypothermia are examples of short and long-term impacts. These effects produce changes in a population/community and therefore can cause modifications in the entire ecosystem [4,5].

It is estimated that 1.3 million tons of oil enter the marine environment every year both from natural sources (cold seeps, releasing low and chronic quantities of hydrocarbons) and from anthropogenic sources, causing a massive oil supply of about 120 million liters per year [6]. Environmental disasters have occurred in recent decades with devastating environmental consequences. Oil undergoes various processes as diffusion, evaporation, dispersion, dissolution, emulsification, photo-oxidation, surfacing, biodegradation. In the biodegradation process, microorganisms present in the marine environment use organic pollutants as the main or only source of carbon and energy by converting them, through a step by step oxidation process, into $CO_2$, $H_2O$ and biomass [7,8]. Biodegradation by microorganisms represents one of the main mechanisms by which oil and other contaminants are removed by marine environment. The release into marine environment of hydrocarbons, due to natural causes, constitutes a minimum percentage, used as a source of nourishment by a wide range of microorganisms, which are therefore capable of carrying out a natural disposal [9].

Oceans are not flooded with oil, testifying the efficiency of microorganisms networks that degrade hydrocarbons, started to reveal how and when they use hydrocarbons as a source of carbon and energy.

## 2. PHYSICAL-CHEMICAL-BIOLOGICAL METHODS OF REMOVING OIL HYDROCARBONS

The interest in the degradation of polluting substances has undergone a considerable increase in recent years for responding to the need in finding new eco-sustainable strategies for environmental remediation. In recent decades, various physical, chemical and biological methods have been proposed for the remediation of marine environment contaminated by oil [10].

The main intervention techniques for removing oil by water can be grouped into:

- *mechanical containment or recovery*: it is generally applied through the use of barriers and skimmers, that remove only the hydrocarbon floating on the water;
- *natural or synthetic absorbent materials*;
- *chemical or biological methods* such as dispersing or gelling agents;
- *physical methods* such as cleaning with absorbent materials or high pressure washing.

Some of these approaches, adopted for the remediation of contaminated marine environment, involve a disposal problem when they use, for example, solvents. Conventional physical-chemical decontamination methods can also be quite expensive and non-ecosustainable. Generally, these treatments should be considered as emergency measures to be used in the initial stages in the event of accidental oil leakage, to quickly check the spread and drift of oil, but are not appropriate for ecological restoration [11].

The limited effectiveness of these traditional treatments stimulated the development of alternative technologies based on biodegradation. The bioremediation represents an efficient, economic and low environmental impact alternative, leading in most cases to the complete degradation of pollutants [12].

## 3. MICROORGANISMS DEGRADING HYDROCARBONS

The presence of oil in the environment for millions years brought to the evolution of microorganisms capable of degrading and using hydrocarbons as the main or exclusive source of carbon and energy. These microorganisms play a fundamental role in keeping ecosystem and biosphere to generate a sustainable environment.

Research conducted in recent decades gave a solid basis for current knowledge about the microbiology of biodegradation of hydrocarbons, allowing for the identification of an increasing number of microorganisms. To date, numerous bacteria, yeasts, fungi and algae from both terrestrial and aquatic ecosystems are reported for their ability to degrade hydrocarbons. The biodegradation return varies by 6% to 82% for fungi, by 0.13% to 70% for soil bacteria and by 0.003% to 100% for marine bacteria [13-16]. Among all microorganisms, bacteria are primary degraders and the most active agents in biodegradation; they can degrade a wide range of hydrocarbon substrates through aerobic and anaerobic pathways, even if the fastest and most complete degradation of most organic pollutants occurs under aerobic conditions, thanks to the metabolic advantage of having the availability of $O_2$ as acceptor of electrons.

Most of these are bacteria belonging to the Proteobacteria phylum (α-, β-, γ-Proteobacteria), and to the order of Actinomycetales (Gram-positive with a high content of G + C). Recently, degraders belonging to the Firmicutes phylum (Bacillus, Geobacillus) and the Deinococcus-Thermus phylum (Thermus) have been isolated. Other bacterial strains belonging to the Bacteroidetes-Chlorobi phylum (Flavobacteria and Sphingobacteria) have been found in oil contaminated environment, suggesting their possible role in biodegradation [17-19].

## 4. MARINE BACTERIA DEGRADING HYDROCARBONS

The most evident proof of the great importance that bacteria have in the deterioration of hydrocarbons comes from marine environment. The capacity to use hydrocarbons as a growth substrate is known for several marine bacterial types, classified as *generalist* and *specialized*:

- *generalist species*: they have a metabolism capable of using, in addition to hydrocarbons, various growth substrates. In this category there are numerous species, belonging to Pseudomonas, Marinomonas and Vibrio types;

- *specialized types*: the HC obliged degrading bacteria, known as hydrocarbons clast (BIC), to which belong the types Alcanivorax, Marinobacter, Thalassolituus, Oleispira, Oleibacter, some members of type Planomicrobium and Cycloclasticus, have a highly specialized metabolism towards hydrocarbons. It is hypothesized that the high specificity of BICs is an adaptive strategy allowing them to survive in a highly oligotrophic environment such as the marine one, so that they are practically not detectable in uncontaminated environment, but they constitute up to 90% of the microbial community near oil spills.

The predominance of a bacterial strain over others varies according to the physical-chemical characteristics of the contaminated environment, according to the concentration of nutrients and to the type of present hydrocarbon. The class of pollutant selects the bacteria capable of using it as a carbon source by activating different catabolic pathways, each of which involves different enzymes and specialized in the degradation of specific substrates [20, 21].

## 5. FACTORS INFLUENCING BIODEGRADATION

The efficiency of biodegradation depends on various factors including the complexity of the substrate to be degraded in terms of molecular weight and structure, and on environmental parameters such as temperature, oxygen, salinity, pressure and nutrients. Generally, the degradation efficiency decreases with the increase of the number of carbon atoms of the HCs; short-chain alkanes are more easily degraded, followed by branched, aromatic, cyclic-aromatic alkanes, asphaltenes and heavy compounds.

Among physical factors, the temperature plays a very important role because it affects both the chemical properties of the pollutant (solubility, viscosity) and the activity of microorganisms. At low temperatures the viscosity increases, while the volatility of low molecular weight hydrocarbons decreases delaying the beginning of degradation. In general, the biodegradation rate decreases with the decrease in temperature [22].

To overtake the low bioavailability of hydrophobic contaminants, microorganisms adopt strategies that decrease the surface tension through the production of surfactants, which act as emulsifying agents favoring the formation of micelles. Biosurfactants are a heterogeneous group of chemical compounds, such as glycolipids, phospholipids, lipopeptides, lipoproteins, neutral lipids, polymeric lipids and other biopolymers, synthesized during the stationary phase of growth of bacteria.

Many hydrocarbon degrading microorganisms produce biosurfactants. Another strategy implemented by bacteria to promote the direct contact with hydrocarbons is to increase the hydrophobicity of the cell surface. This incorporates the synthesis of adhesion structures such as proteins, mycolic acids and other exopolymers [23, 24].

## 6. MICROBIAL INTERACTIONS IN BIODEGRADATION IN SEA

Being oil a complex mixture, the degradation of all its different components requires the presence of a bacterial consortium. When oil is present in the marine environment, there is a

network of direct/indirect cooperative interactions between members of the same community that coexist. These interactions can occur between physically separate species that use soluble or volatile metabolites for the transmission of information, or between species that are in the immediate vicinity, and that associate themselves forming microbial biofilms.

Bioremediation studies focus on individual microorganisms that degrade contaminants; these ones are part of an ecological network, involving direct and indirect interactions with other members of the microbial community. For this reason, in recent years the attention focused on the study of the entire microbial community present in contaminated sites, to obtain a greater understanding of the impact of hydrocarbon contamination on the entire community [25, 26].

## 7. OXIDIZING-HYDROCARBON MICROBIAL COMMUNITIES

The isolation of hydrocarbon-oxidant bacteria in pure culture is a fundamental point in the analysis of microbial communities, because it allows the analysis of the genetic, biochemical-metabolic and physiological characteristics of the isolated strains, helping to elucidate the metabolic pathways and the genetic ground of the degradation of hydrocarbons and their interactions in situ.

For the isolation of hydrocarbon-oxidant bacteria, the used strategy is to set up enrichment crops, inoculating the environmental samples in mineral soils integrated with HC as the only carbon source. The result is the selective proliferation of all bacterial species capable of surviving in the presence of HC and using them for the growth.

One of the main limitations of cultivation methods is that only a minimal percentage of the bacteria present in nature is cultivable, both because the artificial conditions recreated in laboratory do not allow their growth and because different bacterial strains enter into a vital state that cannot be cultivated when they are subject to changes in vital conditions [27].

The development of new culture-independent molecular techniques allows to study the non-cultivable fraction of bacteria, allowing a wider characterization of the microbial communities and of the degrading bacteria of contaminated sites. Molecular biology techniques (gene libraries) and fingerprinting technologies such as PCR-DGGE (Denaturing Gradient Gel Electrophoresis) allow to study the non-cultivable fraction of bacteria, both in terms of phylogenetic diversity and metabolic and functional diversity. While molecular techniques have made possible to increase knowledge on the entire hydrocarbon-oxidant microbial community, on the other hand the isolation of these bacterial strains remains of fundamental importance for the study of degradation mechanisms [28,29].

## 8. INNOVATIVE SYSTEMS FOR BIOREMEDIATION OF CONTAMINED WATER

New mitigation measures are urgently needed for remediation of marine areas contaminated by oil. In recent years, various physical, chemical and biological methods have been proposed for the remediation of marine environment. Among all these remediation strategies, bioremediation represents a promising non-invasive and economic technology that provides a more sustainable restoration of contaminated waters and sediments [30-32].

The success of bioremediation requires a combination of skills coming from different fields such as engineering, biology, biotechnology, biophysics. Environmental biotechnology could provide significant contributions for solving marine pollution problems caused by oil hydrocarbons through the exploitation of marine microbiological resources and new biotechnological tools such as biopolymers. Numerous world programs raised awareness on the importance of microbial communities in marine environmental processes, in particular in the biodegradation of hydrocarbon pollutants by specialized and generalist hydrocartonoclastic bacteria [33-36].

Bioremediation interventions can be realized in the place of contamination (in situ interventions) or in a confined environment such as bioreactors (ex situ interventions). In situ technology is followed when there is no possibility of transferring the polluted matrix, as when the contamination affects a large area. The natural mitigation is linked to the degradation capacity of indigenous microorganisms. The removal of contaminants through natural mitigation takes long time because degrading microorganisms represent only 10% of the total population.

The rise of in situ bioremediation efficiency can be achieved by stimulating the native degrading bacteria through the alteration of physico-chemical parameters of the environment, adding compounds such as nutrients or acceptors of electrons and oxygen (Biostimulation). If the indigenous microbial community is unsuited or ineffective to support degradation, may be appropriate to resort to bioaugmentation, consisting in introducing degrading allochthonous bacteria into the contaminated environment.

To get the bioaugmentation process successful, microorganisms inserted into the polluted environment must be able to degrade the contaminating present component, persist in a foreign environment and to be genetically vital. The result of bioaugmentation relies on the interaction between populations of autochthonous and allochthonous microorganisms, due to rivalry for nutrients [37].

There are several approaches to bioremediation, in particular:

- *waste treatment systems*, based on biofilms adhered to synthetic or natural surfaces, such as biofilters, aerobic and anaerobic granular sludge reactors, are widely used and widespread. Biofilms of degrading bacteria can be artificially created on inert substrates, such as gravel particles or on glass and plastic plates. In biofilms, bacteria enjoy various benefits that facilitate and improve their biodegradation activity;

- *bioremediation based on the immobilization of HC-oxidant bacteria*: numerous strategies are currently implemented to increase the efficiency of bioremediation, such as the method of immobilizing bacterial cells on different types of media, because the immobilization improves the resistance of microorganisms by increasing their metabolic capacity. Immobilization is determined as the limited mobility of microbial cells with the consequent preservation of vitality and catalytic functions.

This process can exploit the ability of microorganisms to create biofilms on the surface of materials. Immobilization reduces the costs of bioremediation processes, ameliorates their efficiency because it eliminates the problem of dispersion and dilution of cells in the environment, guarantees a steady microenvironment for cells and/or enzymes, leading to a greater tolerance to high concentrations of pollutants [38].

There are five main immobilization techniques used in bioremediation processes:

- *adsorption*,

- *bonding on surface* (electrostatic/covalent),
- *flocculation* (natural/artificial),
- *entrapment*,
- *encapsulation*.

- *Adsorption*: the immobilisation of microbial cells and enzymes by adsorption occurs via their physical interaction with the surface of carriers insoluble in water. This method is fast, simple, environmentally friendly, low cost. Adsorption on a medium occurs through the formation of weak bonds [39,40].

- *Bond on surface*: the electrostatic bond on a surface is similar to that of physical adsorption. It requires to wash the surface of support with a buffer solution for getting a hydrophilic surface capable of negatively attracting charged cells or enzymes. The line of action for immobilization is dissimilar in the case of covalent bond, because it demandes the presence of a binding agent. Immobilization can be executed only with chemical activation by means of vectors enriched with bonds of amide, ether, carbamate. This method is mainly employed for the immobilization of enzymes, being binding agents often toxic to cells [41].

- *Trapping in porous matrices*: the trapping of microorganisms is well known and widely used in bioremediation. It is a quick, non-toxic, versatile method. Trapped microorganisms are protected versus environmental factors. The most valuable parameter in the entrapment of microorganisms is the relation between size of carrier pores and size of cells [42,43].

- *Encapsulation*: it is similar to entrapment, but in this case the immobilized particles are divided by external environment with a semi-permeable membrane. The great advantage of this method is the preservation of cells against adverse conditions of outer environment. Due to the limited permeability of membranes, encapsulation is not often used for ex situ bioremediation applications.

- *Carriers for bioremediation*: the immobilization process requires vectors with specific properties, not all materials are in fact suitable for immobilization of bacterial cells. The choice of the carrier is an essential element for the success of the bioremediation intervention. It is important to contemplate the type of process (in or ex situ), the type of pollutant and the capacities of immobilized microorganisms. A good carrier should be insoluble, non-toxic for both immobilized cells and the environment, easily accessible, inexpensive, stable and suitable for regeneration. The substrates used for adsorption/binding on the surface would have a high porosity and be biodegradable [44]. These supports are biodegradable, biocompatible, inexpensive because resulting by waste of the food industry [45]. Their application in bioremediation processes is limited due to poor resistance to biodegradation and stability in a restricted pH range [46].

A valid alternative is represented by polymeric substances of synthetic origin. The most used polymers as sorbents are polypropylene, polyethylene, polyurethane. They are quite efficient, but one of main disadvantages is the non-biodegradability. Although several strategies have been proposed to obtain porous devices capable to meet basic requirements such as biodegradability, great absorption capacity, low costs and good efficiency, there are still many challenges to be faced for overcoming strong limitations such as complex manufacturing paths, environmental incompatibility, scarce recyclability.

For this reasons, in recent years biodegradable polymers aroused a lot of interest for their properties by finding applications in various technologically advanced fields such as the production of biomedical devices, the intensification of bioprocess and waste removal. The

success of porous biopolymers can be attributed to different characteristics, such as the relative ease of production compared to other materials and the high absorbency. In this context, the most commonly used biodegradable synthetic polymers and studied for advanced applications include polylactic acid (PLA), and polycaprolactone (PCL) [47,48].

## 9. MATERIALS AND METHODS

As for usable methods materials, a possible way with respective steps is as follows:

- *Biodegradable carriers*: 3D polycaprolactone (PCL) scaffolds are produced by fusion combining mixing in the molten state and leaching of particulates, without the use of toxic solvents. 3D porous materials are prepared by combining two water-soluble porogens (NaCl, PEG) in the polymer fusion matrix and subsequently leaching the PCL/NaCl/PEG compounds in water [49]. 3D scaffolds have the advantage of being produced in large quantities and have a high lipophilicity.

The main polymer of the matrix is represented by polycaprolacton, while the water-soluble porogens are NaCl and polyethylene glycol (PEG) 2000 particles. The average pore size is between 50-100 μm. The polylactic acid (PLA) and polycaprolacton membranes were synthesized by electrospinning using conventional electrospinning equipment. The polymeric solutions were prepared using 10% by weight of PCL, which was dissolved in dichloromethane (DCM): ethanol (EtOH) (8: 2 vol); 10% by weight of PLA was dissolved in chloroform ($CHCl_3$: acetone (Ac) (2: 1 vol)).

The electrospinning was carried out with a sequence of constant parameters: flow rate = 1 ml/h; distance between the tip of the needle and the collector = 15 cm; powered at high voltage = 15 kV; temperature = 25 °C; relative humidity = 40%. The membranes were dried for at least 2 days under suction hood, in order to remove any residual solvents. The membranes are made up of nanoscale fibers with an average diameter of 1.4 μm.

- *Hydrocarbon-oxidizing strains*: Alcanivorax sp. SK2 and Oleibacter sp. 5 belong to the class of Gammaproteobacteria, are classified as marine bacteria degrading obliged hydrocarbons (BIC) for their ability to use almost exclusively alkanes as source of carbon and energy. Alcanivorax borkumensis SK2 was chosen because it is considered the model system of BICs and degrades almost exclusively linear alkanes, cycloalkanes and isoprenoids. Oleibacter marinus 5 was chosen for the degradative characteristics.

Alcanivorax sp. SK2 and Oleibacter sp. 5, Nocardia sp. SoB and Gordonia sp. SoCg were grown in ONR7a and Bushnell Haas (BH) mineral medium respectively. Both culture vehicles were added with 1% (v/v) (C16) sterile hexadecane or crude oil (0.1%) as the only carbon source. The bacterial cultures were incubated at 30 ± 1 °C for 7 days in a shaker (200 × g).

- *Carrier sterilization*: The 3D scaffolds and the PLA and PCL membranes were sterilized by a 30-minute wash in 70% ethanol. The residual ethanol was eliminated by two 30-minute washes with sterile distilled water. An additional sterilization treatment was carried out by treating the samples with UV rays (wavelength: 253 nm) for 30 minutes.

- *Immobilization of bacterial cells and SEM analysis*: the immobilization of bacterial cells on polycaprolacton 3D scaffolds was carried out by inoculating individual colonies of Alcanivorax SK2 and Nocardia SOB in 3 ml of mineral midst (ONR7a and BH) containing the 3D scaffold of PCL (15 mm × 10 mm) previously immersed in 30 μl of sterilized hexadecane by filtration. The cultures were brooded at 30 ± 1 °C under stirring (200 × g) for 48 hours.

The immobilization on electrospun membranes of PLA and PCL was carried out by inoculating single colonies of Alcanivorax SK2, Oleibacter sp.5 and Gordonia SoCg, Nocardia SOB, in 3 ml of mineral medium (ONR7a and BH respectively) containing a membrane of PLA or PCL (15 mm × 10 mm), previously immersed in 30 µl of sterilized hexadecane by filtration. The cultures were incubated at 30 ± 1 °C for 48, 120, 240 hours under stirring (200 × g). Abiotic controls were prepared under the same conditions without bacterial inoculation.

After the incubation period, the 3D PCL scaffolds, the PLA electrospun membranes and the PCL membranes were taken from the bacterial cultures under sterile conditions and treated as follows for SEM analysis. The supports were washed in PBS 1 for 3 minutes and the cells were secured with 4% glutaraldehyde for 30 minutes at 4 °C. The samples have been dehydrated with a growing ethanol solution at room temperature (15, 30, 50, 75, 100%); each wash was executed for 3 minutes [50]. Bacterial adhesion and proliferation in carrier media was assessed practicing scanning electron microscopy (SEM, Phenom ProX, PhenomWorld) [51].

## 10. OIL DEGRADATION IN MICROCOSM

Bacterial cultures have been prepared by inoculating individual colonies of Alcanivorax sp. SK2, Oleibacter sp. 5 and Gordonia sp. SoCg, Nocardia sp. SoB in 25 ml of mineral medium (ONR7a, BH) added with 0.1% (v/v) of oil. The cultures were incubated at 30 ± 1 °C for 7 days under stirring (200 × g). The cells have been centrifuged at 9000 × g for 10 min, washed with sterile water, resuspended in mineral medium and 1 ml of each bacterial suspension with an optical density value (OD600) 0.1 was inoculated in 100 ml vials containing 30 ml of mineral soil (ONR7a or BH) and a 3D scaffold previously soaked with 30µl of crude oil sterilized by filtration (Arabian Light Crude Oil, ENI SpA). Three sets of microcosms were prepared and incubated at 30 ± 1 °C in an agitator (200 × g) for 2, 4, and 6 days respectively.

The same procedure was followed to set up the microcosms containing PLA and PCL membranes increasing the incubation times to 5 and 10 days, in order to observe an almost complete degradation of the oil. Abiotic controls (in the absence of bacteria) and controls with non-immobilized bacterial cells on the carriers have been prepared in parallel to check the degradation of HC due to purely physical-chemical processes, and that of free cell cultures compared to immobilized cells. Each microcosm has been prepared in triplicate for each condition.

- *GC-FID analysis*: after the carriers removal from each microcosm, the global extracted hydrocarbons (TERHC), in liquid phase, have been analyzed by high resolution GC-FID using the EPA 3510 system (Environmental Protection Agency). After the sample acidification, TERCHs have been extracted at room temperature using dichloromethane ($CH_2Cl_2$, 10% v/v). TERCHs in the carriers have been extracted using hexane ($C_6H_{14}$) as extraction solvent. The extraction work (both for the liquid medium and for the carriers) has been repeated three times and the solvent phase combined and dehydrated with anhydrous sodium sulphate ($Na_2SO_4$).

The extracts have been concentrated by rotating evaporation (Rotavapor) at room temperature. All measurements have been performed using the DANI Master GC (DANI Instruments S.p.A.,) rapid gas chromatography system provided with a split/splitless SSL injector and a FID detector. The samples (1 µl) have been injected in splitless mode at 330 °C. Restek Rxi-5 Sil MS column with Integra-Guard, 30 m × 0.25 mm (internal diameter 0.25 µm)

has been used as analytical column. Helium was used as carrier gas, maintained at a constant flow of 1.5 ml $min^{-1}$. TERCH concentrations were calculated as single concentrations of n-alkanes, pristane and phytane [51].

## 11. CONCLUSIONS

Environmental biotechnologies offer great innovation opportunities that can be reached only by combining skills from different sectors such as engineering, biology, biotechnology, biophysics. It is viable the application of the acquired knowledge and the microbiological resources derived from the exploration of contaminated environments for the development of an innovative system, ready to use, for the bioremediation of water contaminated with oil.

It is of increasing interest the development of new bioremediation systems based on the interaction between biodegradable biopolymers and HC biodegradable bacteria. The challenge is to create a new path of bioremediation by combining the new possibility of biopolymer materials (mechanical removal) with the exploitation of natural marine resources (biological removal), to achieve a greater degradation efficiency of pollutants.

The Blue (or Marine) Biotechnology is based on the exploitation of marine resources for creating products and applications of industrial and environmental interest. Among blue biotechnologies, the bioremediation, exploiting the catabolic potential of microorganisms for the development of processes and products in the treatment and remediation of contaminated areas, is an extraordinary sector of development. In this perspective, environmental microbial biotechnologies offer great innovation opportunities, reachable only by combining skills from different sectors.

The immobilization results a promising factor for biodegradation by suggesting a possible in situ and ex situ application of the created carrier-bacteria system. In general, an increase of between 15% and 20% is observed for immobilized cells compared to planktonic cells. One of the most efficient system seems to be that based on Alcanivorax immobilized on both supports [52-59].

**Biography**

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