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Review Article

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Marine Bioremediation - A Sustainable Biotechnology of Petroleum Hydrocarbons Biodegradation in Coastal and Marine Environments

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Abstract

Bioremediation is now the best successful initiative to mitigate and to recover sites contaminated with hydrocarbons and has been the preferred process for clean-up contamination around the oceans of the world. The advantages of marine microorganisms in the removal of petroleum hydrocarbons, exemplifies the eco-sustainable bioremediation that can be achieved in sensitive marine environments and probably until now the only approach for biodiversity rich and fragile environments. The use of bio-surfactants to protect the marine environment is particularly attractive since a number of marine bacteria and microalgae strains can produce bio-surfactants during growth on hydrocarbons. Moreover, according to recent results, marine microorganisms, exhibit the maximum yield and surface-active property compared to terrestrial species. Because of the interest to find ecofriendly solutions for the bioremediation and biodegradation of petroleum hydrocarbons in the marine environment, the use of marine microorganisms and their respective bio-surfactants is preferable to that non-marine and of synthetic origin. The aim of this review is to integrate the advantages of marine bioremediation coupled to hydrocarbon removal from marine environments. This alternative of bioremediation is a natural process of waste treatment, relatively cost-effective than other remediation approaches that are used for clean-up of hazardous waste in coasts, seas and oceans that can be adaptable to variable environmental conditions, viz, estuarine, coastal and marine pollution and is widely accepted by the society.

Keywords: Bioremediation; Biodegradation; Petroleum, Hydrocarbons; Marine; Bio-surfactant

Introduction

Petroleum-derived products are the major source of energy for industry and societies. The transport of petroleum across the world represents a frequent and potential for oil spills in the marine environment [1]. Actually, it is widely recognized that petroleum hydrocarbons contamination has impacted, and damaged the world oceans, seas and coastal zones and represent a constant threat to the planet Earth health sustainability [2].

The recent catastrophe and sequels remnants after approximately 600,000 tons of crude oil hydrocarbons released by the Deep-water Horizon explosion in the Gulf of Mexico, has increased the volume of petroleum enters the marine environment each year (~ 1.3 million tons). That scenario and the past oil spill accidents in oceans at large scale and the continuous anthropogenic negligence in coastal seas and Bays, has also contributed to renovate the world public awareness on the magnitude of the environmental damage [3,4].

At small scale, productive environments and biota are highly impacted, especially in low-energy habitats, such as lagoons and salt marshes. Marine biotechnology is a technology of the Century to contribute to the sustainable development of our planet. The growing pressure on our natural resources by the increasing population growth and pollution also has impacted the planet on water and land resources. Contaminated coastal and marine environment, generally result from the continuous carelessness and negligence of anthropogenic activities on the former unlimited abundance of land and marine resources of early times [5]. The increasing need to remedy adverse effects of anthropogenic activities on estuarine, coastal and marine ecosystems [6], has prompted the development of effective bioremediation strategies [7]. Nevertheless, probably one of the main interests of bioremediation is its compatibility with the major natural biogeochemical cycles and recycling routes of the earth and marine ecosystems, which make bioremediation a sustainable and environmentally eco-friendly approach for cleanup-polluted environments [8]. Moreover, given the difficulties of the level and the scale in marine areas, very little practical bioremediation and restoration has been carried out for open marine systems [6,9]. The physical, chemical and mechanical technologies to remove petroleum hydrocarbons from contaminated marine environments in most of the cases are unsustainable and can be expensive. Bioremediation is a cost-effective and sustainable biotechnology for the treatment of contaminated coastal and marine sites. This biotechnology utilize living cells or biological components to complete biodegradation of complex organic contaminants to other simpler organic compounds into carbon dioxide, water, inorganic compounds, and cell protein [10,11]. The objective of this review is to update the current status and potentials of marine bioremediation as a sustainable alternative for impacted fragile and sensitive marine environments.

Advantages of marine bioremediation and biodegradation of petroleum hydrocarbons

In marine environment, bioremediation is considered as the most effective and attractive cleaning biodegradation biotechnology to

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decrease the level of pollution and to recover contaminated marine environments [11]. The technology of bioremediation relies on the use of the diverse metabolic capabilities of microorganisms or their parts for the degradation and removal of many environmental pollutants [10,12]. Bioremediation is widely applied due to the increased practical approaches of natural attenuation and biodegradation, in most natural marine sites [11]. The following bioremediation approaches are usually applied in marine environments impacted by an oil spill, (i) addition of oil degrading bacteria to supplement or to enrich the existing microbial biota, which is called bio-augmentation; and (ii) application of fertilizers (nutrients), to encourage and stimulate the growth of indigenous oil degraders, that is named bio-stimulation [10].

The actual most contaminant and emerging problems in polluted saline and marine waters can find solution by bioremediation, *e.g.* aquaculture and fisheries effluents, trace metals, endocrine disrupters, mixed waste/ municipal wastewaters discharges, crude and refined oil pollution, as well as biological carbon sequestration. Bioremediation has now been the best successful initiative to remediate sites contaminated in the sea with hydrocarbons or other specific contaminants and has been the preferred process for clean-up contamination around the oceans of the world. However, under certain critical situations, interfering factors, can reduce the efficient achievement and removal of pollutants. Among these factors, we can list the following: the nature of the contaminant (s), structure, water solubility, bioavailability, biodegradability, co-metabolism potential, substrate/metabolite concentration, and toxicity, the properties of the seawater, nutrients, oxygen, salinity, presence of bioavailability enhancing agents, temperature, pH and, and the activity of the microbial population [13]. The alternative of bioremediation is a natural process of waste treatment widely accepted by the society that can be adaptable to variable environmental conditions, *viz*, estuarine, coastal and marine pollution. The residues of the treatment are usually harmless products and include carbon dioxide, water, and cell biomass [7]; the remained residues of the biodegradation can be recycled, and regenerated into more cell biomass in the respective environments to be implemented (coastal, estuarine, and extreme marine environments). The bioremediation treatment can be performed in conditions with dilute, dispersed or widely diffused contaminants of seawater and can often be carried out *on site*, *in situ*, and *ex-situ*.

In addition, probably most important is it noninvasive nature without causing a major disruption of normal activities. The Bioremediation approaches can prove less expensive, and relatively cost-effective than other remediation approaches that are used for clean-up of hazardous waste in coasts, seas and oceans [7,13].

Biodegradation of petroleum hydrocarbon contaminants in coastal-marine environments

Bioremediation is an efficient degradation technology that is currently used to remove hydrocarbons from contaminated sites, since mechanical, physical and chemical treatments have limited effectiveness [10]. Bioremediation exerts its action on biodegradation. Biodegradation by natural populations of microorganisms represents one of the primary and natural mechanisms by which petroleum and other hydrocarbon pollutants can be processed, bio-transformed and removed from the environment. However, the passiveness and slow action of natural biodegradation in order to be effective require be complemented by other bioremediation measures. In this context, the ability to establish and maintain conditions that favor enhanced oil biodegradation rates in the contaminated environments is a major factor to be considered.

Any biodegradation action in the marine environment must to understand how hydrocarbons are degraded by microorganisms, and thereby mitigate ecosystem damage. It is known that crude oils and refined products are mostly composed of biodegradable molecules, whose decomposition from the environment depends as they are consumed by microbes [14]. Once the hydrocarbons are released in seawater, several processes occur, which can contribute to the bioremediation and biodegradation in the marine ecosystem (Figure 1).

In the case of shorelines, saltmarshes and beaches, successful bioremediation field trials have been reported. At higher oil concentrations, oxygen plays an important role for the achievement of successful bioremediation. When oil had penetrated into the anoxic

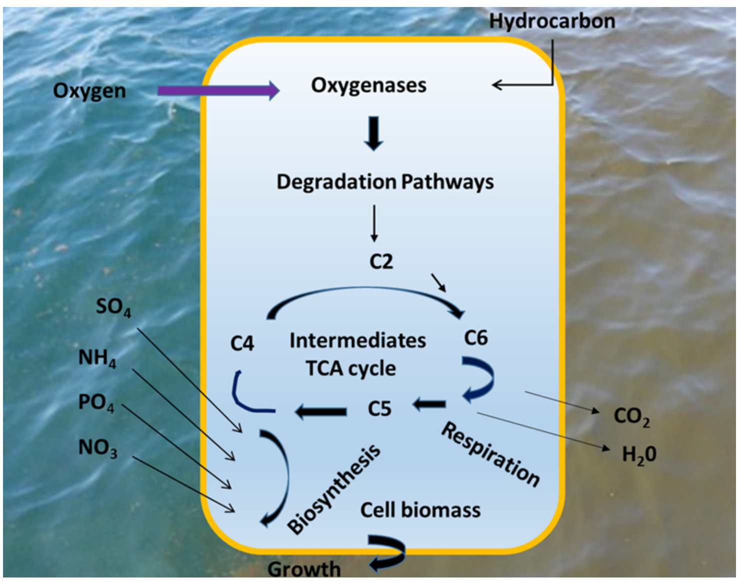

Figure 1: Main mechanisms involved in the aerobic biodegradation of petroleum hydrocarbons in the marine environment (modified after [10]).

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layer of sediments, oxygen additions is a common bioremediation strategy, in order to overcome oxygen levels depletion. In addition, in programs of salt marshes recovery, it may be feasible to bio-remediate oil-contaminated fine sediments by using nutrient and oxygen amendments [15]. Among the complementary programs, additions of lyophilic sources of nutrient may encourage biodegradation on the surfaces of rocks under proper bioremediation schemes. The strategic plan to bio-remediate oil spilled on the shorelines is to treat first the oil that is sorbed to beach sediment. It is suggested that bioremediation approaches works better after physical removal of oil; hence, biodegradation should be focused on the oil remaining on the beach after other processes are largely complete. This approach could be widely applied to different shoreline types. The presence of hydrocarbon degrading microorganisms play an effective role in Bioremediation of any spilled hydrocarbons in the contaminated environment. Hydrocarbon degraders are ubiquitous in the marine environment. The presence of a single microbial species, which can degrade only one or two classes of hydrocarbon within a crude oil, reduce significantly the kinetics of bioremediation [15]. In such circumstances, consortia of microorganisms are required to significantly biodegrade a large fraction of crude oil. In cases where the indigenous microflora is deficient in hydrocarbon-degrading potential, the strategy of bioaumentation can be a good option. Hence, seeding of active degraders will depend of the respective environmental condition. The use of allochthonous microorganisms and its degradative efficiency in the contaminated ecosystem will depend of several factors. For example, the prevailing environmental parameters on the selected site for bioremediation play an important role, *viz*, oxygen, salinity, pH, temperature, nutrient quantity and quality. For instance, environmental conditions related to the site to be remediated plays a key role in the successful program of bioremediation. For instance, pH of the site is an important factor to consider according to the requirements of the added microorganisms (particularly for salt marshes) or adaptations of the organisms to the tidal cycles, the salinity, or the oligotrophic conditions of many beach environments [15].

Keeping aerobic conditions ensure rapid and complete degradation of petroleum hydrocarbons [16] as shown in Figure 2. The initial oxidative process is carried out by oxygen, which activate enzymatic key intracellular reaction by the catalytic action of oxygenates. The different degradation pathways lead to intermediates of the central intermediary metabolism, viz the tricarboxylic acid cycle. Lately, cell growth and biosynthesis of biomass occurs from the central precursor metabolites [10]. Apart of this mechanism, the production of bio- plays also a key role in hydrocarbon biodegradation [17,18] as explained down lines and shown in Figure 3. During the last couple of decades, bioremediation has been part of the programs of effectively restore and recovery of polluted marine sites. The complex composition of crude oil, mostly constituted from thousands of components, *viz*, saturates, aromatics, resins and asphaltenes [1] difficult its treatment. Upon discharge into the sea, hydrocarbons are exposed to weathering, which produces the combined effects of physical, chemical and biological modification. That is why; the first molecules degraded in the marine environments are especially those of smaller molecular weight such as saturates hydrocarbons, which are readily biodegraded. The presences of aromatics compounds with one, two or three aromatic rings, which are abundant in marine environments, are also efficiently biodegraded. Nevertheless, compound's with higher number of aromatic ring (>4) are recalcitrant and quite resistant to biodegradation. In such situation are asphaltenes and resin fractions, which contain higher molecular weight compounds [1]. Nutrients, especially nitrogen and phosphorous plays an important role in hydrocarbon degradation and bioremediation. The quality and quantity of available nitrogen and phosphorus in seawater affects the growth and activities of hydrocarbon-degrading microorganisms mostly in a marine environment. These nutrients acts as fertilizers to an oil-contaminated marine environment and can stimulate the biodegradation of spilled oil, as was the action undertaken in the large-scale application for bioremediation after the oil spill from the Exxon Valdez in Alaska [1]. Despite many microorganisms capable of degrading petroleum components have been isolated, only few of them have been tested and reported as successful when applied for petroleum biodegradation in marine environments. The case of Alcanivorax spp., frequently associated in oil-contaminated marine habitats has been reported as successful in biodegradation studies.

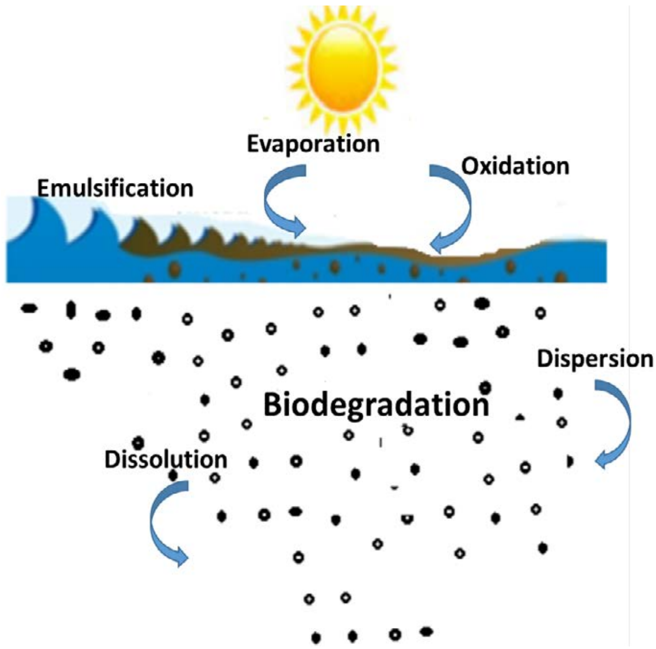

Figure 2: Biodegradation processes of oil in the marine environment.

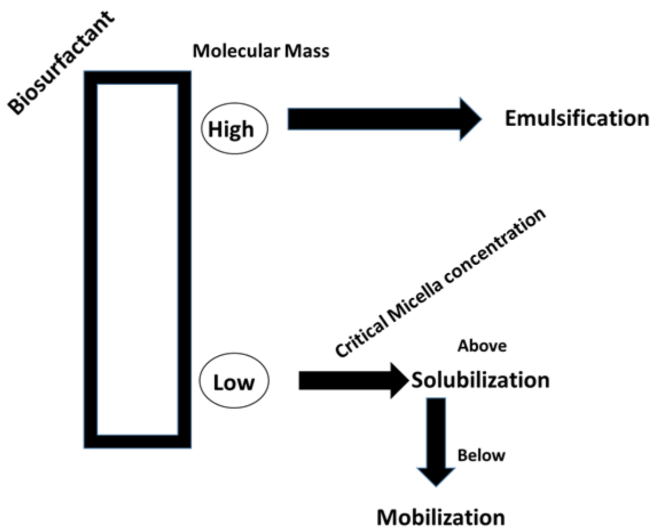

Figure 3: Mechanisms of hydrocarbon removal by biosurfactants depending on their molecular mass and concentration [37].

The fertilization *in situ* by enrichments of nitrogen and phosphorus, undertaken to stimulate the growth of bacteria, also produces at the same time, an increase in the endogenous biodegradation capabilities of petroleum hydrocarbons [1].

Biodegradation of petroleum hydrocarbons in marine environments are influenced by salinity and temperature. These parameters affect the structure and physiology of existing microbial communities and modify the solubility and viscosity of the pollutants. As long as the environmental conditions become extreme, a decrease in the metabolic potential and diversity of degrading bacteria is observed [19,20]. Concerning the structure of the pollutants, a remarkable inhibitory effect of salinity was observed for aromatic than for aliphatic compounds [21]. At salinities higher than 20%, *Marinobacter hydrocarbonoclasticus* degraded various aliphatic and aromatic hydrocarbons [22]. Halophilic *Marinobacter*-dominant culture isolated from an oil production facility in Oklahoma, shown degradation capacities for benzene, toluene, ethyl benzene, and xylenes (BTEX) completely at 14% salinity [23]. The biodegradation of petroleum compounds at different salinities by mat microorganisms of the Arabian Gulf coast of Saudi Arabia showed the bioremediation potential of these mats developed on polluted oil environments. In these conditions, pristine and n-octadecane were optimally degraded at salinities between 5 and 12% (weight per volume NaCl). The optimum degradation of phenanthrene was at 3.5% salinity, and 8% for dibenzothiophene and 28-401°C for both aliphatics and aromatics [19].

Marine bio-surfactants: an effective role in bioremediation and biodegradation of Petroleum Hydrocarbons in Marine environments

The structurally diverse group of surface-active metabolites, synthesized by microorganisms, is classified as bio-surfactants [24,25]. The bio-surfactants produced by some marine microorganisms are promising agents for bioremediation of hydrocarbons, particularly of oil pollution in marine environments [24]. Because of the reduce surface and interfacial tensions exerted by these molecules, in both aqueous solutions and hydrocarbon mixtures, makes them potential candidates for enhancing oil recovery [25], and actually are under intense research, particularly for the bioremediation of the sea polluted by crude oil. The property of microorganisms to accumulate bio-surfactants on the cell surface and adheres to hydrocarbon self-define the microbial cell itself as a bio-surfactant.

Most of the microbial surfactants are lipid in nature and grouped into glycolipids, phospholipids, lip peptides, natural lipids, fatty acids and lipopolysaccharides [26,27]. Bio-surfactants are amphipathic molecules with a hydrophilic and a hydrophobic domain, which facilitate the uptake of hydrocarbons into cells [28]. Bio-surfactants offer attractive properties and products in petroleum-related activities and industries for use in enhanced oil recovery, in cleaning oil spills, in oil emulsification, and in breaking industrially derived oil-in-oil emulsions.

Bio-surfactants exhibit three major characteristics of surface-active agents, such as: (i) enrichment at interfaces, (ii) lowering interfacial tension and (iii) micelle formation. The industrial advantages of these over the chemical surfactants were reported as following: lower toxicity [29], higher biodegradability; better environmental compatibility; high selectivity and specific activity at extreme temperatures, pH and salinity resistant [25].

The use of bio-surfactants to protect the marine environment is particularly attractive since a number of marine bacterial and microalgae strains can produce bio-surfactants during growth on hydrocarbons [17,18]. Moreover, according to Maneerat [24] microorganisms of marine origin, exhibit the maximum yield and surface-active property compared to terrestrial species.

Because of the interest to find ecofriendly solutions for the marine environment, the use of bio-surfactants is preferable to those of synthetic origin. However, scarce information on either bio-surfactant produced by marine microorganisms or bio-surfactants active in saline water has been reported so far. In order to effectively work in removal of petroleum hydrocarbons, the following environmental conditions are aspects to be considered: salinity, pH, temperature and pressure [30,31]. Among microorganisms, only bacteria and microbial consortia have been probed as effective in the practical removal of hydrocarbons from seawater, but the cases of yeasts, algae and protozoa still are under research with highly promising potentialities [17,18,30].

Biodegradation of hydrocarbons by native microbial populations is the primary mechanism by which hydrocarbon contaminants are removed from the environment. Hence, biosurfactants are capable of increasing the bioavailability of poorly soluble polycyclic aromatic hydrocarbons such as phenanthrene [32].

However, the addition of biosurfactants has been reported as stimulant of the indigenous bacterial population to degrade hydrocarbons at rates higher than those which could be achieved through addition of nutrients alone [25]. A successful bioremediation at large scale was reported on the Exxon Valdez oil spill. In such case, the application of Rhamnolipid from *P. aeruginosa*, exhibited significant removal capacities for oil from contaminated Alaskan gravel [33,34]. In another experiment, 56% of the aliphatic and 73% of the aromatic hydrocarbons were recovered from hydrocarbon-contaminated sandy-loam soil by treatment with *P. aeruginosa* biosurfactant [25].

Most of the biosurfactants are anionic or nonionic; the structure is a characteristic of the microorganism producing the surfactant under the specific growth conditions [26,35,36].

In oil- polluted saline environments, biosurfactants from halophilic/halotolerant microorganisms play a significant role in the accelerated remediation of these environments. Probably the major advantage of biosurfactants is their property to induce relaxation or attenuation of surface tension, which increase solubility and enhances degradation of hydrocarbons as exemplified in Figure 3 modified from Pacwa, et al. [37], which exhibit the different mechanisms of hydrocarbon removal by biosurfactants depending on their molecular mass and concentration in aqueous media. Microbial production of a surfactant derived from trehalose by marine species as the case of *Rhodococci* proved successful for n-alkanes removal [38]. These kinds of applications could have important roles as surface-active agents for *in situ* bioremediation of fragile and sensitive environments anchoring a high biodiversity as in the case of marine environments. For instance, strains able to degrade n-alkanes (C10–C30) in the presence of 30% (w/v) NaCl actually are a reality [18]. Moreover, brines removal from industrial activities (agricultural, wastewater treatment-desalinization, salt production, etc.) can be successfully treated with the use of exopolysaccharides (EPS) produced and supported by marine organisms as well as with the use of heterotrophic and halophilic bacteria for the treatment of hyper saline wastewaters. A main and surrogated role of EPS in Cyanobacteria and certain microbial consortia and organisms from coastal – marine environments have been reported as able to metabolizing oil hydrocarbons, a condition that is enhanced by pollutant substrate as hexadecane [17]. The mentioned advantages of biosurfactants produced by marine microorganisms in the removal of petroleum hydrocarbons, exemplifies the eco-sustainable bioremediation that can be achieved in sensitive marine environments and probably until now the only approach for biodiversity rich and fragile environments.

Conclusions

From the analysis of this review, we conclude that bioremediation is an effective eco-friendly treatment tool for the cleaning of certain oil-contaminated estuaries, shoreline, seas and oceans. Because of the natural processes that are encouraged by bioremediation, ensures a lower environmental impact compared with mechanical, physical and chemical removal approaches of oil in the sea. It is expected that combined and integrated studies on microbial populations and respective production of biosurfactants will enhance the biodegradation approaches of spilled-oil at the sea. The complex composition and toxicity of the oil spill could be attenuated by reducing and converting the many components from hydrocarbons into innocuous and recyclable products such as carbon dioxide, water, and biomass. New innovative bioremediation products which are tailored to specific contaminated environments are required as well as degradative microbial strains, specifically designed to biodegradate or detoxification of pollutants in saline environments. A well designed microbial consortium will have complementary catabolic pathways, as well as the potential to disperse and make the hydrocarbons readily bioavailable. Therefore, novel microorganisms should be bio-prospected and screened for bioremediation and biodegradation approaches on complex mixtures of pollutants without causing adverse effects. The possibilities of production of biosurfactants from microorganisms grown on petroleum hydrocarbons will effectively improves the bioremediation potentials on oil pollutants, particularly oil polluted in marine environment and exemplifies the eco-sustainable bioremediation that can be achieved in sensitive marine and fragile environments.

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