

Bioremediation strategies for oil polluted marine ecosystems

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Abstract

The marine environment is subject to contamination by organic pollutants from a variety of sources. Significant degradation of these compounds may take many years and it is frequently necessary to consider methods that can accelerate this process. While seeding with oil degraders have not convincingly demonstrated to be effective, addition of nitrogenous fertilizers has been documented to have increased rates of petroleum biodegradation. The prospects for enhanced biological degradation of petroleum organic compounds are reviewed in this paper. A conceptual framework that can be adapted for the development of techniques for dealing with oil pollution will be discussed. These include physical, chemical, and biological methods. A number of different technologies may fall into the category of biological methods. They include the use of straw or plant material as an absorbent for oil, biosurfactants to clean oiled surfaces, and the addition of materials to encourage microbiological biodegradation of oil. The present review addresses the progress that has been made in each of these aspects with particular emphasis on bioremediation.

Keywords: Marine; Organic pollutants; biodegradation; Nitrogenous fertilizers; cometabolism; biosurfactants.

Abbreviations: HOCs-Hydrophobic organic contaminants; NAPL-Non-aqueous- phase liquid; CMCs-Critical micelle concentrations; PAHs- Polyaromatic hydrocarbons; N:P-Nitrogen:Phosphorus; USEPA- United States environmental protection agency.

Introduction

Oil is a complex and variable natural substance. Its fate and effects depend on the type and quantity of oil spilled, properties of the oil as modified over time by physical and chemical processes, the organisms and habitats exposed, and the nature of the exposure. Crude oils and refined products are principally hydrocarbons, and the vast majority of these are biodegradable (Prince, 2002). The greater the complexity of the hydrocarbon structure, i.e. the higher the number of methyl branched substituent's or condensed aromatic rings, the slower the rates of degradation and the greater the likelihood of accumulating partially-oxidized intermediary metabolites (Atlas, 1995). Nevertheless, while hydrocarbons are an excellent source of carbon and energy for the microbes that consume them, they are incomplete foods in that they do not contain significant concentrations of other nutrients, such as nitrogen and phosphorus, which are required for microbial growth (Prince, 1993). Consequently, a significant spill usually overwhelms the natural supply of these nutrients in the environment, and biodegradation quickly becomes nutrient limited. The bioremediation of oil spills in aerobic marine environments has therefore focused on adding these limiting nutrients to at least partially overcome the nutrient limitation, while at the same time minimizing any additional impact of the fertilizer addition (Prince, 2002). Depending on the degree of an intervention, bioremediation is generally considered to include natural attenuation, bio-stimulation (addition of nutrients, and electron donors/acceptors to promote the growth or metabolism of certain micro-

organisms) or bio-augmentation (the deliberate addition of natural or engineered micro-organisms with the desired catalytic capabilities) (Singer et al., 2005; Van Dillewijn et al., 2007). Selecting response options, including physico-chemical, seeding, nutrient addition and natural recovery, involves considering tradeoffs among their potential environmental impact, appropriateness for habitat, and application timing (USEPA, 2001). As marine environment is subject to contamination by organic pollutants from a variety of sources, organic contamination therefore results from uncontrolled releases from manufacturing and refining installations, spillages during transportation (Atlas, 1995), direct discharge from effluent treatment plants and run-off from terrestrial sources (Ubalua, 2007). In quantitative terms, crude oil is one of the most important organic pollutants in marine environments and it has been estimated that worldwide somewhere between 1.7 and 8.8 x 10⁶ tons of petroleum hydrocarbons impact marine waters and estuaries annually (National Academy of Sciences, 1985). When oil is spilled, about 25% of it is evaporated within days depending on the prevailing temperature. Oil has effect on the marine life no matter where it is spilled. The amount of light that enters the water is reduced by 90% causing many marine plant and protist growth to decrease because of the reduced rate of photosynthesis. Oil also decreases the amount of oxygen that dissolves in water. Although bacteria are the most active agent of bioremediation, fungi and other strong oxidative enzymes are key players in recycling recalcitrant

polymers and xenobiotic chemicals as well (Atlas, 1995). Many plants, natural and transgenic associated to rhizosphere micro-organisms are extraordinarily active in removing or immobilizing pollutants (Kuiper et al., 2004; Ryan et al., 2008). Biodegradation of different classes of petroleum compounds occurs simultaneously, but at different rates. The rate of biodegradation of oil depends not only on the type of oil and the specific microbial population, but also on the environmental conditions, such as free or dissolved oxygen, optimum temperature for oil degradation (20-35°C), physical and/or chemical dispersion of oil, turbulent conditions as opposed to quiescent conditions, and low concentrations of organic matter. Oxygen and nutrients are believed to be an important limiting factor affecting biodegradation not only in marine environment, but also in fresh water system (Ubalua and Ezeronye, 2005). Thus, oxygen availability is needed to support microbial degradation of hydrocarbon-pollutants while nutrients (nitrogen and phosphorus) stimulate the microbial degradation of hydrocarbons in polluted marine ecosystems. Petroleum bioremediation is carried out by microorganisms capable of utilizing hydrocarbons as a source of energy and carbon (Rosenberg et al, 1998; Rosenberg and Ron, 2000). Microbial utilization of hydrocarbons is highly dependent on the chemical nature of the hydrocarbon compounds in petroleum mixtures and on the environmental conditions, as well as bacterial species (Atlas, 1995). Common oil degraders found in marine environments are: *Pseudomonas*, *Achromobacter*, *Flavobacterium*, *Cinetobacter*, *Vibrio*, *Bacillus*, *Arthrobacter*, *Norcardia*, *Micrococcus*, *Corynebacterium*, as well as fungi, algae and yeast (Watanabe, 2001). It has been long-known that ecological systems possess a degree of innate capacity for breaking down pollutants or contaminants that enter them (Ogbuehi et al., 2011). The biological agents responsible for much of this self-cleansing are most often microbial in nature. Microbial mediated removal of contaminants from natural systems overtime can occur entirely in the absence of human intervention. The process can also be given a “jump start” through human management or resource input. One biological strategy that can enhance contact between bacteria and water-insoluble hydrocarbons is emulsification of the hydrocarbon. Therefore, it is not surprising that bacteria growth on petroleum usually produce potent emulsifiers. These surfactants help to disperse the oil, increase the surface area for growth, and help detach the bacteria from the droplets after the utilizable hydrocarbon has been depleted (Rosenberg, 1993). Researches on cost-effective, environmentally benign clean-up strategies were prompted by spill incidents. Physical and, on rare occasions, chemical methods are capable of rapidly removing the majority of oil, but they are rarely completely successful (Prince, 1997). Natural degradative processes aid in removing the remaining oil. Bacteria are considered to represent the predominant agents of hydrocarbon degradation in the environment (Leahy and Colwell, 1990), and hydrocarbon-degrading bacteria are ubiquitous. As a single species typically is capable of degrading only a limited number of the compounds found in crude oil, a consortium composed of many different bacterial species is usually involved in oil degradation. Crude oil typically contains high carbon content and the low level of other nutrients essential for microbial growth, and as such the rate and extent of degradation are in general limited by the low availability of nitrogen and phosphorus (Prince, 1997). Consequently, growth of hydrocarbon-degradation bacteria and hydrocarbon degradation can be strongly enhanced by fertilization with inorganic nitrogen and phosphorus. The Exxon Valdez spill

formed the basis for a major study on bioremediation through fertilizer application and was the largest application of this emerging technology. Inipol (an oleophilic microemulsion with urea as a nitrogen source, laureth phosphate as phosphate source, and oleic acid as a carbon source) and customblen (a slow-release fertilizer composed of calcium phosphate, ammonium phosphate and ammonium nitrate within a polymerized vegetable oil coating) were used (Atlas, 1995). This has proven an effective bioremediation treatment on several types of shorelines (Bragg et al., 1994; Venosa et al., 1996; Swannell et al., 1996; Swannell et al., 1999). Bioremediation studies have, in general been dominated by an empirical approach and optimum nutrient amendment levels are often informed by laboratory incubations. In the field, care must be taken in supplying optimum concentrations of inorganic nutrients. Too high concentrations may result in eutrophication, and too low concentrations may result in sub-optimal biodegradation. However, bioremediation has shown promise as a potentially effective and low-cost treatment option, but concerns about the slow process rate and bioavailability limitations have hampered more widespread use of this technology.

Oil clean-up strategies

Strategies are conceptual plans designed to achieve response objectives as depicted in Fig 1. As information is gathered, strategies can be developed (and revised) to protect those resources at risk. Though response strategies will vary according to incident-specific conditions, strategies can often be established in spill response planning consistent with response goals (USEPA, 2001). In such a scenario communities exposed to hydrocarbons become adapted, exhibiting selective enrichment and genetic changes that result in increased proportions of hydrocarbon degrading bacteria and bacterial plasmids encoding hydrocarbon catabolic genes (Atlas and Atlas, 1991). Arguably, low temperature will generally increase oil viscosity, inhibit spreading, evaporation, and emulsification, and may extend response windows of opportunity (but some oils may form stable emulsions in low temperatures). During extreme cold weather, ice may limit the spread of oil and improve the chances of recovery or burning. In high temperature and humidity situations, oil will spread and evaporate faster, increasing fire and explosion potential, accelerating weathering processes, reducing response windows, and impacting equipment deployment times (USEPA, 2001). Reducing overall environmental consequences in an effective and efficient manner usually requires a combination of techniques, including:

- Natural recovery;
- Physical washing;
- Physical removal
- Physical in-situ treatment (including burning); and
- Chemical or biological treatment (USEPA, 2001)

Oil accidentally released in the marine environment can have severe negative effects on natural systems. Spills do degrade on their own over time as a result of several natural physical, chemical and biological “weathering” processes. The time it takes for an oil spill to breakdown and dissipates naturally depends on several factors, including the volume of the spill and the physical characteristics of the crude, the weather, sea state conditions, and whether the spill remains at sea or is carried onshore, particularly in environments sheltered from wave action, the process can be prolonged. The truth is that

many crude oil spills can persist within the environment for some time after the spill, breaking up and dissipating slowly and often requiring an active clean-up response. Physical/or mechanical clean-up strategies to contain and recover oil from large spills are typically pursued first, possibly followed by chemical strategies that can speed the dispersion and dilution of the contaminating oil. Overtime, oil hydrocarbons can be partly or completely mineralized. Marine microbial flora with oil-degrading capabilities includes species of bacteria, fungi and yeasts. Such organisms have been found to reside within the water column, sediments, and even in sea ice (<http://www.marinebiotech.org/biorem.html>). A key appeal of biotech-based bioremediation in coastal systems is that it can be incredibly cost effective compared to alternative clean-up strategies. The speed and efficiency with which bioremediated contaminants are broken down depends primarily on three variables: temperature, the concentration of potentially limiting nutrients and the amount of oxygen available in the environment.

Physico-chemical methods

Petroleum products are subjected to various weathering process that alter their chemical and physical nature on entering the marine environment. The chemically and biologically induced changes in the composition of a polluting petroleum hydrocarbon mixture are known collectively as weathering. Microbial degradation plays a major role in the weathering process. Biodegradation of petroleum in natural ecosystems is complex. The evolution of the hydrocarbon mixture depends on the nature of the oil, on the nature of the microbial community, and on a variety of environmental factors which influence microbial activities (Atlas, 1995). The fate of hydrocarbons in petroleum/oil can be summarized as photochemical reaction; photo-oxidation, evaporation and biodegradation (Atlas, 1995). Hydrocarbon biodegradation can occur over a wide range of temperatures, and psychrotrophic, mesophilic, and thermophilic hydrocarbon-utilizing microorganisms have been implicated. Thus ZoBell (1973) and Traxler (1973) reported on hydrocarbon degradation at below 0°C; Klug and Markovetz (1967) and Mateles et al. (1967) reported on hydrocarbon degradation at 70°C. Expectedly, temperature can have a marked effect on the rates of hydrocarbon degradation, though the effect differs depending on the hydrocarbon composition of a petroleum mixture. Low temperatures retard the rates of volatilization of low-molecular-weight hydrocarbons, some of which are toxic to microorganisms. The presence of such toxic components was found to delay the onset of oil degradation at low temperatures. Empirical data has shown that at 20°C, lighter oils had greater abiotic losses and were more susceptible to biodegradation than heavier oils; and in contrast rates of oil mineralization for the heavier oils were significantly lower at 20°C than for the lighter ones. The light crude oils, however, had toxic volatile components which evaporated only slowly, inhibiting microbial degradation of these oils at 10°C (Atlas, 1995). It is therefore suggestive that the influence of temperature on hydrocarbon degradation is more complex than simple consideration. Thus the effects are interactive with other factors, such as the quality of the hydrocarbon mixture and the composition of the microbial community. Temperature often is not the major limiting factor for hydrocarbon degradation in the environment except as it relates to other factors such as the physical state of the oil or whether liquid water is available for microbial growth. Though biological

approach is less cumbersome and cost effective, physical/ or mechanical alternatives may become imperative in the event of large magnitude spillage in order to short-circuit time that will be required for intrinsic bioremediation. In large accidental spills, physical/ or mechanical methods followed by chemical approach are always the first method of choice. The approach involves scooping, the use of skimmers and controlled burning. Usually, skimmers are attached to ships and once the oil has been picked up, it is separated from the water inside the ship. Materials such as straw, powdered clay, saw dust, chopped corncobs stuffed in cloth "sausages", and other organic and inorganic absorbents are used to contain the oil in areas where they can be skimmed. Such methods can offer an appreciable oil clean-up of only 10 to 30%. Subsequently, chemical dispersants such as detergents and solvents are used to get rid of the oil, but these chemicals are toxic to bottom-dwelling (benthic) organisms, shore-dwelling organisms, and open water marine life (<http://www.marinebiotech.org/biorem.html>).

Biological methods

Bioremediation or biorecovery is a managed or spontaneous process in which microbiological processes are used to degrade contaminants to less toxic or non toxic forms, thereby remedying or eliminating environmental contamination. Microorganisms depend on nutrients and carbon to provide the energy needed for their growth and survival. Degradation of natural substances in the marine ecosystems provides the necessary food for the development of microbial populations. Bioremediation technologies harness these natural processes by promoting the enzymatic production and microbial growth necessary to convert the target contaminants to non toxic end products (Atlas, 1995). The biodegradation of petroleum in the marine environment is carried out largely by diverse bacterial populations, the most important (based on frequency of isolation) genera of hydrocarbon utilizers in aquatic environments are *Pseudomonas*, *Achromobacter*, *Arthrobacter*, *Micrococcus*, *Nocardia*, *Vibrio*, *Acinetobacter*, *Brevibacterium*, *Corynebacterium*, *Flavobacterium*, *Candida*, *Rhodotorula*, and *Sporobolomyces* (Atlas and Bertha 1992). Bacteria and yeasts appear to be the prevalent hydrocarbon degraders in aquatic ecosystems. In polluted freshwater ecosystems, bacteria, yeasts, and filamentous fungi all appear to be important hydrocarbon degraders (Cooney and Summers, 1976). Comparatively bacteria and yeasts exhibit decreasing abilities to degrade alkanes with increasing chain length. In contrast filamentous fungi does not exhibit preferential degradation for particular chain lengths. However, patterns of degradation, i.e., which hydrocarbons could be utilized, were similar for bacteria and fungi, but there is considerable variability among individual organisms. It therefore suggests that the ability to utilize hydrocarbons is widely distributed among diverse microbial populations. Hydrocarbons are naturally occurring organic compounds, and it is not surprising that microorganisms have evolved the ability to utilize these compounds. When natural ecosystems are contaminated with petroleum hydrocarbons, the indigenous microbial communities are likely to contain microbial populations of differing taxonomic relationships which are capable of degrading the contaminating hydrocarbons. Thus, the hydrocarbon-biodegrading populations are widely distributed in the world's oceans. Surveys of marine bacteria indicate that hydrocarbon-degrading microorganisms are ubiquitously distributed in the marine environment (Atlas, 1995). Many of these microorganisms possess the enzymatic

capability to degrade petroleum hydrocarbons. Some degrade alkanes, others aromatics, and others both paraffinic and aromatic hydrocarbons. Often the normal alkanes in the range C₁₀ to C₂₆ are viewed as the most readily degraded, but low-molecular-weight aromatics, such as benzene, toluene and xylene, which are among the toxic compounds found in petroleum, are also very readily biodegraded by many marine microorganisms. The greater the complexity of the hydrocarbon structure, the slower the rates of degradation (Atlas, 1995). The major metabolic pathways for hydrocarbon biodegradation are well known. The initial steps in the biodegradation of hydrocarbons by bacteria and fungi involve the oxidation of the substrate by oxygenases, for which molecular oxygen is required. Alkanes are subsequently converted to carboxylic acids that are further biodegraded via β -oxidation (the central metabolic pathway for the utilization of fatty acids from lipids, which results in formation of acetate which enters the tricarboxylic acid cycle). Aromatic hydrocarbon rings generally are hydroxylated to form diols; the rings are then cleaved with the formation of catechols which are subsequently degraded to intermediates of the tricarboxylic acid cycle. Interestingly, fungi and bacteria form intermediates with differing stereochemistries. Fungi, like mammalian enzyme systems, form *trans*-diols, whereas bacteria almost always form *cis*-diols (many *trans*-diols are potent carcinogens whereas *cis*-diols are not biologically active). Since bacteria are the dominant hydrocarbon degraders in the marine environment, the biodegradation of aromatic hydrocarbon therefore results in detoxification and does not produce potential carcinogens. The complete biodegradation (mineralization) of hydrocarbons therefore produces the non-toxic end products carbon dioxide and water, as well as cell biomass (largely protein) which can be safely assimilated into the food web (Atlas, 1995). While various physico-chemical processes have been developed for treating these pollutants, these approaches are often prohibitively expensive, non-specific or have the potential for introducing secondary contamination. As a result, there has been an increased interest in eco-friendly bio-based treatments commonly known as bioremediation. To date cometabolism is now regarded as one of the most important mechanisms utilized by microorganisms for the transformation of chlorinated aliphatic and aromatic contaminants (Bouwer and Zehnder, 1993). It is a process whereby microorganisms involved in the metabolism of a growth promoting substrate also transform other organic contaminants (cosubstrates) that are not growth supporting if supplied as sole carbon and energy source. It is an important process in both aerobic and anaerobic environments. Consequently, compounds which otherwise would not be degraded can be enzymatically attacked within the petroleum mixture due to the abilities of the individual microorganisms to grow on other hydrocarbons within the oil (Horvat, 1972). A petroleum hydrocarbon mixture, with its multitude of potential primary substrates, provides an excellent chemical environment in which co-oxidation can occur. Many complex branched and cyclic hydrocarbons undoubtedly are removed as environmental contaminants after oil spills as a result of co-oxidation (Perry, 1979). Hence co-oxidation was hypothesized to be responsible for the degradation of a number of compounds in the oil to account for the similar rates of disappearance of compounds which are normally resistant. Assessing the role of co-oxidation in natural environments is difficult since multiple microbial populations are present. In mixed-population studies, synergism could be an alternative hypothesis to explain the observed results.

The use of straw or plant materials

Sorbents are used to remove surface oil with oleophilic (oil-attracting) material placed in water or at the waterline. They include sausage boom, pads, rolls, sweeps, snares, and loose granules or particles. These products can be synthetically produced or can be natural substances (USEPA, 2001). The capacity of the material fibers to remove crude oil from marine ecosystems may be related to the chemical composition and surface properties of the fibers as well as the concentration, specific gravity and temperature of the crude oil. In addition, the amount of oil sorbed increases as the temperature decreases. The presence of these sorbent materials (straw or plant materials) in an oil spill area facilitates a change of phase from liquid to semi-solid. Once this change is achieved, the removal of the oil together with the sorbent structure becomes less difficult. Often sorbents are structured into sheets, booms, or pads, which are reusable and easier to control. In oil spill clean-up operation hydrophobicity and oleophilicity are primary determinants of successful sorbents. Other important factors include retention of oil over time, recovery of oil from sorbents, amount of oil sorbed per unit weight of sorbent and reusability and biodegradability of sorbent (Melvold et al., 1988). Oil sorbent materials can be categorized into three major classes; inorganic mineral products, organic synthetic products and organic vegetable products (The International Tanker owner pollution Federation Limited, 1980). Mineral products includes: perlite, vermiculites, sorbent clays and diatomite (Melvold et al., 1988). These materials do not show adequate buoyancy retention and their oil sorption capacity is generally low. Among synthetic products, polypropylene and polyurethane foam are the most widely used sorbents in oil spill clean-up because of their highly oleophilic and hydrophobic properties (Melvold et al., 1988; The International Tanker Owner Pollution Federation Limited, 1980). A disadvantage of these materials is that they degrade very slowly as compared with mineral or vegetable products. It has been reported that organic vegetable products such as straw, corncobs and wood fibre showed poor buoyancy characteristics, relatively low oil sorption capacity, and low hydrophobicity (Melvold et al., 1988). These natural sorbents have hydrophobic properties before any treatments due to their surface waxes. Milkweed floss especially is expected to have high oil sorption capacity because of its high wax content and its hollow structure. Precisely, one gram of milkweed floss was observed to sorb approximately 40 g of light crude oil at room temperature. Therefore, the exceptionally high oil sorption by milkweed fibre can be explained by the large amount of wax on the fibre surface, approximately 3% compared to the 0.4 – 0.8% wax content of cotton, and the larger and non-collapsing lumen of the fibre, which gives more void volume for absorbed oil (Knudsen, 1990). Comparatively, Kenaf fibre is much coarser than cotton and milkweed, but similar to polypropylene. It occurs in a fibre bundle rather than a single fibre. Further separation of this fibre bundle to single fibres should increase oil sorption comparably to polypropylene (Röling et al., 2002). Below are a number of methods that can be adapted for oil spill clean-up both on-and-offshore (Table 1). Increase of oil sorption at low temperatures is associated with increase in oil viscosity at these temperatures. However, the rate of oil penetration into a capillary is inversely proportional to the oil viscosity and directly proportional to the capillary radius due to hydrophobic interactions and van der Waals forces between the crude oil and the wax in the natural sorbents. Therefore, an increase in oil viscosity reduces the rate of

Table 1. Physical, mechanical and biological methods of oil spills clean-up strategies

Rapid Removal of oil from Ecosystem
Physical removal
Floating sorbent + mechanical harvesting
Sorbent attached to revolving drums or belts
Gelling agent + mechanical harvesting
Mechanical skimming
Suction pumping + separation
Revolving metal drums
Controlled combustion
Glass beads
Particulate silicas
Slow Removal of Oil from Ecosystem
Dispersion + biodegradation
Sinking sorbent + biodegradation
Biodegradation promoters

Modified from Johnson et al., 1973

Physical/or mechanical clean-up



Chemical strategies



(Biological methods)
(a) Straw/plant materials



(b) Biosurfactants



(c) Nutrients addition

Fig 1: Schematic representation of a typical oil polluted marine ecosystem clean-up strategies

absorption within the internal capillary movement of oil, but at the same time it increases the adherence of oil onto the surface of the fibre and within the capillary during drainage (Röling et al., 2002). This phenomenon however, appears to be more significant in light crude oil due to its low viscosity.

Biosurfactants

A number of hydrocarbon-degrading microorganisms produce emulsifying agents and some of these bioemulsifiers have been considered for use in oil cleaning operations. Improvements in biodegradation rates have been achieved by the addition of these surfactants to the contaminated environment. Banat et al., (2000), described biosurfactant to be amphiphilic molecules consisting of a hydrophilic part and a hydrophobic part. They proposed that as a consequence of this property, these molecules tend to concentrate at

interfaces thereby decreasing interfacial tension. It has been established that above a certain concentration (critical micelle concentration), stable aggregates of 10 to 200 molecules are formed (called micelles) which can increase the solubility of hydrophobic organic contaminants (HOCs) (Edwards et al., 1991). Biosurfactants of diverse chemical nature and molecular size are produced by the hydrocarbon-degrading microorganisms. These surface-active materials increase the surface area of hydrophobic water-insoluble substrates and increase their bioavailability thereby enhancing the growth of bacteria and the rate of bioremediation. The effect of surfactants on the availability of organic compounds can be explained by three main mechanisms: dispersion of non-aqueous-phase liquid (NAPL) organics, leading to an increase in contact area caused by a reduction in the interfacial tension between the aqueous phase and the non-aqueous phase; increased apparent solubility of the pollutant

caused by the presence of micelles that contain high concentrations of HOCs (Edwards et al., 1991), and facilitated transport of the pollutant from the solid phase, which can be caused by lowering of the surface tension of the soil particle pore water, interaction of the surfactant with solid interfaces, and interaction of the pollutant with single surfactant molecules (Maakar and Rockne, 2003). Biosurfactants can enhance growth on bound substrates by desorbing them from surfaces or by increasing their apparent water solubility (Marcoux, 2000). Surfactants that lower interfacial tension dramatically are particularly effective in mobilizing bound hydrophobic molecules and making them available for biodegradation. One of the major reasons for the prolonged persistence of high molecular weight hydrophobic compounds is their low water solubility which increases their sorption to surfaces and limits their availability to biodegrading microorganisms. When organic molecules are bound irreversibly to surfaces, biodegradation is inhibited (van Loosdrecht, 1990). Low molecular weight biosurfactants that have low critical micelle concentrations (cmcs) increase the apparent solubility of hydrocarbons by incorporating them into the hydrophobic cavities of micelles (Makkar and Rockne, 2003). Perhaps a more promising solution for bioavailability enhancement may be the use of biosurfactants, whose primary function is to facilitate microbial life in environments dominated by hydrophilic-hydrophobic interfaces (Barkay et al., 1999). Biosurfactants have the ability to solubilize HOCs but have several additional advantages over synthetic surfactants that make them superior candidates in bioremediation schemes (Makkar and Cameotra, 2002; Desai and Banet, 1997). First, biosurfactants are readily biodegradable and therefore pose no additional pollution threat and secondly, most studies indicate that they are completely non toxic to microorganisms and therefore unlikely to inhibit biodegradation of PAHs. Biosurfactant production is potentially less expensive than synthetic surfactants and can be easily achieved in situ at the contaminated sites from inexpensive raw materials. They are more effective than synthetic surfactants in increasing the bioavailability of hydrophobic compounds. In addition, they are selective, environmentally friendly and generally less stable than most synthetic surfactants (Ron and Rosenberg, 2002). For example, high molecular weight bio-emulsifier alasan was recently shown to significantly increase the rate of biodegradation of several PAHs by increasing their apparent solubilities 5-20-fold (Rosenberg et al., 1999 and Barkay et al., 1999). Several biosurfactants have found applications in environmental remediation. They include some small well-characterized biosurfactants such as *Pseudomonas aeruginosa* rhamnolipids (Providenti et al., 1995; Maier and Soberon-Chavez, 2000), *Candida apicola* sophorose lipids (Hommel et al., 1994), *Rhodococcus erythropolis* trehalose dimycolipids (Kanga et al., 1997), *Bacillus* sp. lichenysins (Jenny et al., 1991) and *Bacillus subtilis* surfactin (Makkar and Cameotra, 1997, Awashti et al., 1999). These are potent surfactants, as they reduce surface tension from 72 to less than or equal to 30 dynes/cm and have low cmcs. Bacteria make low molecular weight molecules that efficiently lower surface and interfacial tensions and high molecular weight polymers that bind tightly to surfaces (Desai and Banat, 1997; Rosenberg and Ron, 1997 and Rosenberg et al., 1999). Low molecular weight biosurfactants are generally glycolipids in which carbohydrates are attached to a long-chain aliphatic acid or lipopeptides. One of the best studied glycolipids is rhamnolipid, produced by several species of *Pseudomonas*. The high molecular weight bacterial surfactants are produced by a large number of bacterial

species from different genera and are composed of polysaccharides proteins, lipopolysaccharides, lipoproteins or complex mixtures of these biopolymers. They are less effective in reducing interfacial tension but are efficient at coating the oil droplets and preventing their coalescence. They also exhibit considerable substrate specificity. There are at least two ways in which biosurfactants are involved in bioremediation. First is by increasing the surface area of hydrophilic water-insoluble substrates and secondly by increasing the bioavailability of hydrophobic compounds. For bacteria growing on hydrocarbons, the growth rate can be limited by the interfacial surface area between water and oil (Ron and Rosenberg, 2001). When the surface area becomes limiting biomass increases arithmetically rather than exponentially. If emulsion occurs at, or very close to the cell surface and no mixing occurs at the microscopic level, then each cluster of cells creates its own microenvironment and no overall cell-density dependence could be expected (Ron and Rosenberg, 2002). In effect, the "emulsifier" frees the cell to find fresh substrate. At the same time the depleted oil droplets has been "marked" as used, because it now has a hydrophilic outer surface to which the bacterium cannot attach. The detachment of bacteria from the depleted oil droplets enables them to move to other drops where they metabolize the specific group of utilizable hydrocarbons. Therefore, detachment of bacteria from oil drops results in a more efficient bioremediation. Oil-degrading bacteria can utilize only a limited group of hydrocarbons; hence bacteria attached and growing on an oil droplet become nutrient-starved once this group of hydrocarbons is depleted. If the biosurfactant is cell-bound it can cause the microbial cell surface to become more hydrophobic, depending on its orientation (Ron and Rosenberg, 2002).

Optimizing bioremediation performance through nutrients addition

Serious accidental oil spills in the marine environment have provided the impetus for large-scale bioremediation experiments involving addition of nutrient cocktails to marine and foreshore environments (Prince, 1992; Atlas and Bartha, 1992; Pritchard et al., 1992). Studies on nutrient addition have provided convincing evidence that this is an efficacious approach to bioremediation of oil pollutants in marine environments. It has been demonstrated to overcome a critical rate-limiting factor in aerobic oil-contaminated marine environments. Rates of stimulation have therefore been typically about 3-5 times the natural rates of oil biodegraded. Greater stimulation might be achieved by higher levels of nutrient addition, but this could risk ecological side effects such as toxicity to marine life and eutrophication with associated algal blooms (Atlas, 1995, and Ubalua and Ezeronye, 2007). The use of Inipol EAP22 (oleophilic micro-emulsion with urea as a nitrogen source, lauric phosphate as a phosphate source, and oleic acid as a carbon source) and customblen (slow-release calcium phosphate, ammonium phosphate, and ammonium nitrate within a polymerized vegetable oil coating) was approved for shoreline treatment and was used as a major part of clean-up effort. Consequently remarkable results were observed on the shorelines treated with Inipol EAP22 and customblen compared to non-bioremediated shorelines (Pritchard et al., 1992). Hence bioremediation treatments are aimed at stimulating pollutant-degrading microorganisms to speed the recovery of contaminated ecosystem to a pre-pollution state in terms of biodiversity and ecosystem function (Röling et al., 2002). Although there is evidence that bioremediation can

be used to treat oil-contaminated marine ecosystems effectively, an important limitation of the technology is the difficulty in formulating treatment strategies that will produce a specified outcome in terms of degradation rate and residual contaminant concentration (Head, 1998). Clearly, there is little consensus on how to best optimize nutrient amendment but typically values of around 1-5% N by weight of oil have been used with a ratio of N:P between 5 and 10:1 (Head, 1998). The three types of fertilizers investigated were: water-soluble nitrogen and phosphorus; slow-release isobutylene diurea; and oleophilic Inipol EAP 22, which contains lauryl phosphate and urea. Oleophilic fertilizer application was followed by dramatic cleansing of the shorelines within a few weeks. Extensive efficacy testing indicated that fertilizer application stimulated the rate of microbial hydrocarbon degradation. Testing for toxicity and ecological effects showed minimal toxicity which was restricted to the immediate region of fertilizer application, and does not result in algal blooms (Atlas and Atlas, 1991). The majority of bioremediation strategies aimed at ameliorating marine oil spills assume that the principal mechanism of hydrocarbon removal is aerobic respiration. Whereas this may be valid for oil spills on coarse pebble or cobble shorelines, oxygen availability is likely to assume greater importance in beaches with fine-grained sediments, such as mudflats or salt marshes.

Conclusions

The physical state of petroleum hydrocarbons has marked effect on their biodegradation and their degradation from oil spills seem to be highly dependent on localized environmental conditions. The ability of microorganisms to degrade hydrocarbons is being taken advantage of in major bioremediation efforts. Seeding with microbes, nutrient addition and oxygen supplementation have all been used in bioremediation efforts, with the most convincing demonstrations of bioremediation efficiency resulting from fertilizer and oxygen treatments. Of significance, however, is the fact that human intervention can enhance and accelerate the bioremediation process. If intrinsic bioremediation rates are too slow, for example, it may be possible to manipulate environmental conditions to stimulate contaminant biodegradation by the natural microbial community. One difficulty is that bioremediation is carried out in the natural environment which contains diverse uncharacterized organisms and another difficulty is that no two environmental problems occur under completely identical conditions, for example, variations occur in the types and amounts of pollutants, climate conditions and hydro-geodynamics. These difficulties have caused the bioremediation field to lag behind knowledge-based technologies that are governed by common rationales (Watanabe, 2001). Despite the growing acceptance of bioremediation as a means to treat spilled oiled in marine environments the mechanisms that promote the process under field conditions remain poorly constrained. Although general statements can be made regarding the enhancement of biodegradation by nutrient amendment, there is no consensus on how to best optimize nutrient additions. Careful application of fertilizers can stimulate oil biodegradation two- to five-fold, with no adverse environmental impacts. Such stimulation may seem rather small, but can translate to oil being removed months to years earlier than it would otherwise have been removed.

Bioremediation therefore, may make a significant contribution towards accelerated habitat recovery by the removal of toxic hydrocarbons components to levels below the toxicity threshold. It is thus an important part of toolkits available for dealing with accidental and deliberate oil releases into the marine environment (Prince, 2002).

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