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REVIEW PAPER

Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes

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Abstract Bioremediation, involving bioaugmentation and/or biostimulation, being an economical and eco-friendly approach, has emerged as the most advantageous soil and water clean-up technique for contaminated sites containing heavy metals and/or organic pollutants. Addition of pre-grown microbial cultures to enhance the degradation of unwanted compounds (bioaugmentation) and/or injection of nutrients and other supplementary components to the native microbial population to induce propagation at a hastened rate (biostimulation), are the most common approaches for in situ bioremediation of accidental spills and chronically contaminated sites worldwide. However, many factors like strain selection, microbial ecology, type of contaminant, environmental constraints, as well as procedures of culture introduction, may lead to their failure. These drawbacks, along with fragmented literature, have opened a gap between laboratory trials and on-field application. The present review discusses the effectiveness as well as the limitations of bioaugmentation and biostimulation processes. A summary of experimental studies both in confined systems under controlled conditions and of real case studies in the field is presented. A comparative account between the two techniques and also the current scenario worldwide for in situ biotreatment using bioaugmentation and biostimulation, are addressed.

Keywords Bioremediation · Contaminants · Crude oil · Biodegradation

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Introduction

The world demand for oil in 2008 was 85.62 million barrels per day (OPEC 2009). The global transport and use of both petroleum and its derivatives have made petroleum hydrocarbons (PHCs) major contaminants in both prevalence and quantity in the environment.

Several components of PHCs are biodegradable, but some are recalcitrant whilst others have intermediate biodegradability, depending on their chemical structure and physical state. Crude petroleum is mainly composed of linear and branched-chain alkanes, cycloalkanes and aromatics, but it also contains small amounts of oxygen-, nitrogen- and sulfur-containing compounds, such as phenol, indole and thiophene, respectively. Alkenes are present in several refined petroleum products but usually not in crude petroleum, being mainly obtained by catalytic cracking at high temperatures, whilst the heavy asphaltenes are present in crude but not in refined fractions (Matar 1992).

Although *n*-alkanes are the most biodegradable PHCs, those with 5–10 carbon atoms are inhibitory to most hydrocarbon degraders because they can disrupt the lipid membrane of microorganisms (Bartha 1986). On the other hand, PHCs with C20–C40, usually called waxes, are hydrophobic solids at physiological temperatures. This probably explains their low biodegradability (Bartha and Atlas 1977). Interestingly, some bacteria also produce waxes when they degrade crude oil (Ishige et al. 2003; Walker and Colwell 1976).

The biodegradability of the oil components generally decreases in the following order: *n*-alkanes $>$ branched-chain alkanes $>$ branched alkenes $>$ low-molecular-weight *n*-alkyl aromatics $>$ monoaromatics $>$ cyclic alkanes $>$ polycyclic aromatic hydrocarbons (PAHs) $\gg$ asphaltenes (van Hamme et al. 2003). Bioremediation of these toxic and xenobiotic compounds has gained deserved attention during the last decades, mainly because of the accidental spills and leakages occurring at different locations worldwide (Swannell et al. 1996). Even if a decontamination process is carried out soon after a spill occurrence, the persistence of oil may continue even for decades in shorelines, as it was demonstrated by some well studied oil spill cases, threatening the inhabiting species (Owens et al. 2008). During accidental spills, removal or remediation of the contaminants is usually taken into account right away. However, in the case of petrol stations, refineries and storage tanks, leakage may be small, but continuous and prolonged in time. In this case, the probability of contamination of the groundwater is higher, thus making efficient remediation desirable and needed.

Microorganisms, owing to their biodiversity and vast catabolic potential, have been enormously harnessed for the biodegradation of toxic pollutants since long time (for a comparison between chemical and biotechnological processes see Dua et al. 2002). These diverse catabolic activities are due to the presence of catabolic genes and enzymes (de Carvalho and da Fonseca 2005; Khomenkov et al. 2008; van der Geize and Dijkhuizen 2004). Furthermore, microbes possess other adaptation strategies such as the ability to modify the cellular membrane to maintain the necessary biological functions (de Carvalho et al. 2009; Isken and de Bont 1998), the production of surface active compounds as biosurfactants (Ron and Rosenberg 2002) and of the use of efflux pumps to decrease the concentration of toxic compounds inside the cells (Isken and de Bont 1998; Van Hamme et al. 2003). All these mechanisms and metabolic abilities make microorganisms an interesting and sound tool for the bioremediation of contaminated sites.

Several bacterial strains have been reported to possess the metabolic routes required for the degradation of recalcitrant compounds. Species of *Pseudomonas*, *Mycobacterium*, *Haemophilus*, *Rhodococcus*, *Paenibacillus* and *Ralstonia*, are some of the most extensively studied bacteria for their bioremediation capability (Farhadian et al. 2008; Haritash and Kaushik 2009; de Carvalho et al. 2005). These strains are able to degrade petroleum hydrocarbons and aromatic hydrocarbons such as benzene, toluene, ethylbenzene and xylene (*Pseudomonas*, *Rhodococcus* and *Ralstonia*), as well as polyaromatic hydrocarbons such as naphthalene (*Pseudomonas*), phenanthrene (*Pseudomonas* and *Haemophilus*), anthracene (*Rhodococcus*), pyrene (*Haemophilus* and *Mycobacterium*) and the highly carcinogenic benzo[a]pyrene (*Rhodococcus* and *Mycobacterium*).

To improve the bioremediation process, besides a competent microbe able to degrade the contaminant carbon source, other parameters must be taken into account e.g. water, oxygen, and utilizable nitrogen and phosphorous sources (Rosenberg et al. 1992). Lack of any of the mentioned parameters makes the remediation process under natural conditions inefficient. Strategies involving the addition of seeded cultures, bioaugmentation, or the addition of nutrients, biostimulation, hold the promise of fostering degradation rates (Atlas 1995; Jimenez et al. 2006; Pritchard et al. 1992). Bioaugmentation or seeding is the addition of highly concentrated and specialized populations (single strains or consortia) to the site contaminated with recalcitrant toxic compounds (Leahy and Colwell 1990; Gentry et al. 2004). This technique is best suited for sites that (i) do not have sufficient microbial cells or (ii) the native population does not possess the metabolic routes necessary to metabolize the compounds under concern. On the other hand, biostimulation involves the identification and adjustment of factors such as nutrients that may be looming the biodegradation rate of the contaminants by the indigenous microorganism at the affected site (Swannell et al. 1996). Besides the type and concentration of nutrients, physical and environmental parameters also influence the mineralization rate of hydrocarbons by degrading bacteria. These factors include the chemical composition, physical state and concentration of the crude oil or hydrocarbons; along with the temperature, oxygen availability, salinity, pressure, water activity and pH on the site (Leahy and Colwell 1990).

The decision to implement either or both of these techniques for bioremediation largely depends on the degrading capability of the indigenous microbes and the extent of contamination of the site to be treated. As several examples show, successful laboratory studies concerning bioremediation do not necessarily lead to reproducible in situ decontamination (Balba et al. 1998; Cassidy et al. 1996; El Fantroussi and Agathos 2005). This impending gap between laboratorial trials and on-field studies may be due to several factors influencing the remediation process. Among these are strain selection, indigenous microbial ecology, type of contaminants, environmental constraints, and the procedures used for the introduction of the remediation agents. This review discusses the recent developments in the bioaugmentation and biostimulation strategies for mediated remediation and their potential as on site remediation technologies.

Bioaugmentation and biostimulation: current strategies

Bioaugmentation

A successful bioremediation program usually requires the application of strategies customized for the specific environmental conditions of the contaminated site. The most commonly used options for bioaugmentation are: addition of a pre-adapted pure bacterial strain; addition of a pre-adapted consortium; introduction of genetically engineered bacteria; and addition of biodegradation relevant genes packaged in a vector to be transferred by conjugation into indigenous microorganisms (El Fantroussi and Agathos 2005). The present review will focus on the introduction of single strains and bacterial consortia as a bioaugmentation tool.

Feasibility studies are a prerequisite for any planned intervention that usually revolves around screening, followed by tailoring of a competent microbial formula for a particular site. The initial screening/selection step should be based on the metabolic potential of the microorganism and also on essential features that enable the cells to be functionally active and persistent under the desired environmental conditions. The best approach for selecting competent microbes should be based on the prior knowledge of the microbial communities inhabiting the target site (Thompson et al. 2005; van der Gast et al. 2004). In the case of co-contaminated sites, e.g. contaminated with both high metal concentrations and organic pollutants, the microbial population ability to degrade the organic compounds may be inhibited by the co-contaminants (Roane et al. 2001). The proposed strategies, in such cases, have involved the use of multi-component systems such as a microbial consortium, which is a better representation of a real environment than models based on single-component systems (Ledin 2000).

From an applied perspective, using a microbial consortium rather than a pure culture for the bioremediation is more advantageous as it provides the metabolic diversity and robustness needed for field applications (Rahman et al. 2002a, b; Nyer et al. 2002). Alisi et al. (2009), successfully obtained complete degradation of diesel oil and phenanthrene; a reduction of 60% of isoprenoids; and an overall reduction of about 75% of the total hydrocarbons in 42 days, using a microbial formula made with selected native strains (Alisi et al. 2009). Similarly, Li et al. (2009) affirmed that indigenous microbes could degrade PAH in aged contaminated soil. Albeit, adding microbial consortia (five fungi: *Phanerochaete chrysosporium*, *Cuuninghamella* sp., *Alternaria alternate* (Fr.) Keissler, *Penicillium chrysogenum*, and *Aspergillus niger*; and three bacteria: *Bacillus* sp., *Zoogloea* sp., and *Flavobacterium*) enhanced the degradation rate significantly (41.3%) (Li et al. 2009).

The amount of biomass to be used as inoculum for bioaugmentation is produced in bioreactors, although the transference of such cultures to the site is often critical. Microbial inoculants are homogeneous cell suspensions produced under optimum conditions which often undergo stress when in contact with the complexity of the natural habitats. In real cases, the introduced population starts decreasing shortly after being added due to several abiotic and biotic stresses. The stresses that hamper microbial growth may include fluctuations or extremes in temperature, water content, pH, depletion of nutrients, and also potentially toxic pollutant levels in the contaminated soil (Gentry et al. 2004). Goldstein et al. (1985) found that microbes having potential to degrade organic pollutants in cultures failed to do the same in natural systems. The suggested possible reasons for bioaugmentation failure were: problems concerning the adaptation of the inoculated microorganisms; insufficiency of substrate; competition between introduced and indigenous biomass; use of other organic substrates in preference to the pollutant; and predation (grazing by protozoa) (Goldstein et al. 1985). Thus seeding alone is generally not enough and should be accompanied by suitable physical and environmental alterations (Leahy and Colwell 1990). Factors influencing the efficiency of bioaugmentation and biostimulation are summarized in Table 1.

The use of carrier materials often provides a physical support for biomass, along with a better access to nutrients, moisture and aeration, which extends the survival rate of the microbes (Mishra et al. 2001). Microbial cell encapsulation or immobilization may offer a better survival rate by shielding cells under stressed environmental conditions, usually enabling a faster and more efficient biodegradation as compared to free living cells (Moslemy et al. 2002; Obuekwe and Al-Muttawa 2001). Encapsulation controls the flow of nutrients, lowers the concentration of toxic compounds in the microenvironment of the cells, minimizes cell membrane damage as it reduces the exposure to the toxic compounds and protects from predation and competition; thereby mimicking a miniature bioreactor in the environment (McLoughlin 1994). Several materials like agar, agarose, alginate, gelatin, gellan gum, kappa-carrageenan, acrylate copolymers, polyurethane, and polyvinyl alcohol gel have been well studied and tested to encapsulate or immobilize cells (Cassidy et al. 1996). Moslemy et al. (2002) encapsulated an enriched bacterial consortium (isolated from a gasoline-polluted site) in gellan gum microbeads. Encapsulated cells exhibited a shorter lag phase and thus a higher gasoline degradation rate as compared to their free cell counterparts at equivalent microbial concentrations (Moslemy et al. 2002). In a recent study, Liu et al. (2009) compared the biodegradation of phenol by free and immobilized cells of *Acinetobacter* sp. XA05 and *Sphingomonas* sp. FG03 strains collected from activated sludge and phenol-contaminated soil. They found that the mixture of the two strains performed better than the pure cultures; and immobilized cells performed better in what concerned the degradation of phenol at concentrations higher than 500 mg/l and could be used for 20 cycles (Liu et al. 2009).

Biostimulation

It has been shown that the degradation of petroleum hydrocarbons by a given native microbial population can be favoured by the presence of the required nutrients in the contaminated site (Delille et al. 2004). Spilled petroleum hydrocarbons represent a substantial C-source for the indigenous microorganisms, whereas, in most environments, the presence of

Table 1 Factors influencing bioaugmentation and biostimulation processes

Factors	Description	Reference
Loss of microbial viability during inoculation	Stress due to drastic changes in environmental conditions	vanVeen et al. (1997), Goldstein et al. (1985)
Eventual cell death after inoculation	Depletion of nutrients or toxicity caused by contaminants	Goldstein et al. (1985), Liu et al. (2009)
Competition	Competition for nutrients by autochthonous microbes	Thompson et al. (2005), El Fantroussi and Agathos (2005)
Predation	Bioaugmentation followed by disappearance of bacterial population and protozoa overgrowth	Bouchez et al. (2000)
pH	Extremes in pH inhibit microbial degradation processes	Dibble and Bartha (1979)
Temperature	Temperature influences microbial growth and degradation potential, and also the physical nature and chemical composition of oil	Atlas (1981)
Moisture	Low moisture content limits microbial growth and metabolism; higher values reduce soil aeration	Dibble and Bartha (1979), Leahy and Colwell (1990)

nitrogen and phosphorous is limited. Thus, biostimulation accelerates the decontamination rate, as the addition of one or more rate-limiting nutrients to the system improves the degradation potential of the inhabiting microbial population (Nikolopoulou and Kalogerakis 2009; Prince 1997). Environmental conditions can also be improved to get optimal values of e.g. pH, and moisture content, to achieve optimum microbial degradation conditions (Atlas 1981). Addition of N and P to enhance nutrient level has been studied by several researchers. Sarkar et al. (2005) enhanced the biodegradation of petroleum hydrocarbons by up to 96% after the addition of biosolids (nutrient-rich organic matter resulting from the treatment of domestic sewage) and inorganic fertilizers (rich in N and P) to diesel contaminated soils (Sarkar et al. 2005). Similar results were obtained for diesel oil remediation in the Antarctic coastal sea by biostimulation using a commercial fertilizer (Delille et al. 2009). In the case of marine environments, the addition of biostimulants is more critical, as firstly they should target the microbes near the oil droplets in the seawater and secondly they should not readily get diluted and washed out by the wave action. Moreover, higher concentrations of N and P sources can cause eutrification, thereby enhancing algal growth and ultimately reduce the dissolved oxygen concentration in the water (Nikolopoulou and Kalogerakis 2009).

Besides nutrients there are several other factors that largely determine the PHCs degradation rate under natural conditions. For instance, it was observed that factors including the intensity of physical mixing, the pre treatments (for example shore washing, manual removal, manual raking, bioremediation using biostimulation agents, mechanical tilling, mechanical relocation), and the availability of alternative carbon sources influenced the mineralization potential of the microbes after the Exxon Valdez oil spill (Sugai et al. 1997). Another parameter that has a considerable effect on biodegradation is temperature, due to its effect on the viscosity, water solubility and chemical composition of the oil. It also influences the rate of hydrocarbon metabolism and the composition of the microbial community (Atlas 1981). Mulkins-Phillips and Stewart (1974) studied the effect of temperature (ranging from 5 to 28°C) on the degradation of bunker C fuel oil following the spillage at Chedabucto Bay, using enriched mixed microbial cultures. After incubation for 7 days at 15°C, 41–85% of benzene-soluble components disappeared, whereas, 21–52% degradation was obtained after 14 days of incubation at 5°C (Mulkins-Phillips and Stewart 1974). Horel and Schiewer (2009) studied the impact of temperature and moisture on biostimulation of syntroleum (synthetic diesel oil) using fertilizer as stimulating agent. The bioremediation process started much earlier at a higher than at a lower temperature (20 and 6°C, respectively), for short incubation periods (4–6 weeks). Nevertheless, microbes adjusted to the lower temperature during long incubation periods (12–17 weeks) and degraded up to 50% syntroleum. Nutrient supply was found to be essential for microbial degradation under all conditions in the soil. The degradation of the contaminant after 17 weeks was almost 3 times higher at 20°C and 8 times higher at 6°C when compared to nutrient-deficient sands. However, moisture content (2–12% by weight) and regular mixing to enhance soil porosity did not influence degradation significantly (Horel and Schiewer 2009). On the contrary, it was found that temperature showed limited influence on petroleum degradation for the Antarctic seawater samples in a laboratory microcosm study, where commercial fertilizer enhanced bioremediation (Delille et al. 2009).

Inadequate bioavailability of the hydrocarbons to microorganisms due to low water solubility has been addressed as a limiting step in biodegradation. The use of biosurfactants to enhance the biodegradation rate has been well studied (Rahman et al. 2002a, b; Bordoloi and Konwar 2009; Ron and Rosenberg 2002). Nikolopoulou and Kalogerakis showed that biostimulation using a N and P fertilizer together with biosurfactants enabled naturally occurring microbes to adapt better and faster to the oil spill contamination, ensuring a relatively shorter lag phase and faster degradation rates (Nikolopoulou and Kalogerakis 2008). Apparently, the combination of bioaugmentation, biostimulation, and biosurfactant addition, depending on the characteristics of the contaminated site, might be a promising strategy to speed up bioremediation (Baek et al. 2007). However, any such planned intervention must be followed by ecotoxicity and quality studies of the contaminated site to ascertain that it has regained its natural biological activity and integrity (Hamdi et al. 2007; Liu et al. 2010). These toxicity tests and biological activity measurements should be used as monitoring tools or bioindicators during and after bioremediation of contaminated soils. Such studies provide a better insight into ecological assessment of bioremediation at anthropogenic waste sites and may support decisions for on-site amendments towards a successful bioremediation (Hankard et al. 2004).

Bioaugmentation versus biostimulation

There is a mixed debate on which of the two techniques, bioaugmentation or biostimulation, is a better strategy for bioremediation. Apparently, the circumstances prevailing at the site influence significantly the choice of the technique to be applied. Hamdi et al. (2007) found that selectivity and specialization of added microorganisms mainly defines the bioremediation efficacy, rather than the nutrient load (Hamdi et al. 2007). Bento et al. (2005) compared bioremediation of diesel oil by natural attenuation, biostimulation and bioaugmentation. They concluded that the best approach for bioremediation of diesel oil was the bioaugmentation performed by inoculating microorganisms pre-selected from a contaminated site. Apparently, indigenous microbes (pre-selected for bioaugmentation) are more likely to survive and propagate when reintroduced into the site, as compared to transient or alien strains to such a habitat (Bento et al. 2005; Thompson et al. 2005). On the contrary, there are reports of microcosm and field studies where inoculation with enriched cultures originating from the site itself did not affect hydrocarbon removal rates whereas stimulation was effective (Thomassin-Lacroix et al. 2002). Biostimulation can provide suitable nutrients and conditions for both indigenous and exogenous microbes. Thus, biostimulation becomes a viable approach in those cases where microbial population gets acclimatized due to exposure to hydrocarbons at contaminated sites. Eventually, adapted populations exhibit higher remediation rates than those with no contamination exposure history and thus biostimulation could be sufficient in those cases (Simon et al. 1999; Leahy and Colwell 1990). However, natural acclimatization by the indigenous microbial population often requires a longer time due to an extended lag phase leading to prolonged bioremediation processes (Lendvay et al. 2003).

Due to limitations associated with bioaugmentation and biostimulation when applied individually, these techniques are emerging as complementary. Hamdi et al. amended aged PAH contaminated soil using both bioaugmentation and biostimulation and obtained higher PAH dissipation rates, especially for anthracene and pyrene, than those observed in unamended PAH-spiked soils (Hamdi et al. 2007).

Commercial products: ongoing scenario

Bioaugmentation and biostimulation may contribute to overcome a critical bottle neck of the bioremediation technology. The booming up of bioaugmentation and biostimulation technologies as preferred in situ remediation techniques has attracted commercial interest. Emerging formulations and products are gaining attention and application, claiming fast decontamination rates. Significant enhancement in biodegradation process can be achieved by using different types of bioaugmentation and biostimulation products (Aldrett et al. 1997). However, these products are not panacea and need to be evaluated according to the requirements of the site before implementation. Dott et al. (1989) compared the biodegradability of fuel oil using the commercial product DBC^{TM} (containing nine different dried bacterial mixed cultures) and a mixed culture from a domestic treatment plant during a 31-day incubation period. They asserted that the native microorganisms possessed sufficient biodegradation capability due to eventual adaptations and therefore the rate and extent of biodegradation was higher than that of highly adapted commercial microbial cultures (Dott et al. 1989). Later in 1991, Venosa et al. conducted a detailed study for the screening of ten commercial products (eight bacterial cultures and 2 non-bacterial products) from ten different companies for the bioremediation of samples from Prince William Sound, Alaska. Their results suggested that the degradation of the hydrocarbons could be primarily ascribed to the activity of Alaskan microorganisms (Venosa et al. 1992). Similarly, Simon et al. (2004) did not find any statistical enhancement in the biodegradation rate coefficient after using two commercial bioaugmentation products for removal of petroleum from a wetland (Simon et al. 2004). The metabolic activity, adaptability and ecological competence of commercial inoculants appear to be a major limitation for a successful tailor made remediation. Such products will require a high level of robustness to be exposed to diverse environments. However, enriched

Table 2 Commercial bioaugmentation and biostimulation products

Product	Composition	Results	Reference
DBC-plus^{TM}	Dried bacterial mixed cultures	Depended on the cultures used	Dott et al. (1989)
Biosolve	Anionic biodegradable synthetic surfactant	Reductions of 66% observed for total petroleum HCs during the first 134 days	Sanscartier et al. (2009)
S-200	Oleophilic fertilizer (containing urea, phosphoric esters, oleic acid, water and a solvent)	30% of linear alkanes and aromatics after 60 days	Gallego et al. (2007)
Surfactant AB01039	Mixture of nonionic, biodegradable surfactants	40% of global hydrocarbon depletion after 30 days	Gallego et al. (2007)
Inipol EAP 22	Oleophilic fertilizer containing N source, oleic acid carrier, tri(laureth-4) phosphate and butoxy ethanol	Enhanced biodegradation of the oil two-fold relative to untreated controls	Pritchard et al. (1992)
TerraZyme^{TM}	Microbiological culture	The area of oil covering the rocks reduced from 91.0 to 13.7%	Tsutsumi et al. (2000)

cultures of selected indigenous microbes could produce a more appropriate and cost-effective product for local conditions than commercial bioremediation products (Mohammed et al. 2007). Some examples of commercial bioremediation agents/products and their formulations are shown in Table 2.

Confined systems and real-case studies: bridging the gap

A successful process in the laboratory under controlled conditions does not imply similar success in an uncontrolled environment (Mueller et al. 1992). Indeed it is a pioneering step forward to address potential problems in the environment (Goldstein et al. 1985). Bioaugmentation and biostimulation studies comprising laboratory, simulated field and in situ tests, are very few so far. This type of study can provide insight about the microbes and their growth requirements, before any on-site intervention for decontamination is carried out. Rosenberg et al. (1992), after optimizing conditions for bioremediation of crude oil using a combination of bioaugmentation and biostimulation technique in the laboratory, successfully implemented the same for field and in situ beach remediation (Rosenberg et al. 1992). Gallego et al. (2007) carried out laboratory, pilot, and full-scale experiments to select nutrient sources, surfactants, and other bioremediation amendments for in situ bioremediation of spilled oil. They found that bioremediation can improve the kinetics of the natural processes involved in remediation, thus removing significant amount of toxic fuel oil (Gallego et al. 2007). Some field and oil spill remediation studies using bioaugmentation and biostimulation are presented in Table 3.

Future perspectives and concluding remarks

Bioremediation is a more eco-friendly, cost-effective and efficient environmental clean-up technique when compared to chemical treatment or physical removal of hazardous compounds from contaminated soil or water. Despite some contradictory results from different research groups, bioaugmentation and biostimulation hold the promise of epitomizing in situ bioremediation. Although distinct from each other in principal, the two can be used as complementary techniques for the remediation of oil spills and chronically contaminated sites. The existence of competent microorganisms for a given bioremediation, their nutrient requirements (e.g. carbon, nitrogen, phosphorous, oxygen or an alternative electron acceptor) along with suitable growth conditions (temperature, redox potential, salinity, pH, etc.) should be firstly determined by laboratory and field trials. Such knowledge would allow the manipulation of environmental factors that may limit or prevent the biodegradation of petroleum for in situ applications. Based on these considerations, a deeper understanding of microbial degradation abilities, together with their metabolic networks as well as their cellular resistance and adaptation mechanisms, will bring out a variety of

Table 3 Bioremediation using bioaugmentation and biostimulation: field and real case studies

Technique used	Contaminants/remarks	Location	Reference
Field trials			
Bioaugmentation and biostimulation with inorganic mineral nutrients and/or microbial inoculation	Light crude oil contaminating a sandy beech	Delaware, USA	Venosa et al. (1996)
Biostimulation using forced aeration and nutrient supplementation	Spill simulation (light crude oil and fuel oil) at mature mangroves	Gladstone, Australia	Duke et al. (2000)
Biostimulation by slow release of fertilizer Inipol EAP-22 and 3 different fish compost	Crude oil degradation under severe sub-Antarctic conditions	The Grande Terre, Kerguelen islands	Delille et al. (2002)
Biostimulation using N and P fertilizer	Oil degradation on an coastal salt marsh	Nova Scotia, Canada	Garcia-Blanco et al. (2007)
Biostimulation using fertilizers (urea and diammonium phosphate) and surfactant; moisture content and temperature amendments	Petroleum hydrocarbons degradation in polar desert	Tanquary fiord, high Arctic	Sanscartier et al. (2009)
Biostimulation using manure	Oily sludge-contaminated soil with total petroleum hydrocarbon (TPH) and PAHs	China	Liu et al. (2010)
Oil spills and bioremediation strategy applied			
Bioaugmentation using commercial microbial culture	Heavy crude oil degradation	Nakhodka oil spill, Japan	Tsutsumi et al. (2000)
Bioaugmentation and biostimulation using hydrophobic fertilizer (F-1) as N and P source along with enriched microbial culture	Crude oil degradation	Zvulon beach oil spill, Israel	Rosenberg et al. (1992)
Biostimulation using oleophilic fertilizer	Oil biodegradation	Exxon Valdez oil spill, Alaska, USA	Pritchard et al. (1992)
Biostimulation using liquid inorganic and slow release mineral fertilizer	Crude oil and heavy fuel oil degradation	Sea Empress oil spill, UK	Swannell et al. (1999)
Biostimulation using oleophilic fertilizer	High molecular weight *n*-alkanes, alkylcyclohexanes, and benzenes, and alkylated PAHs	Prestige oil spill, Spain (2006)	Jimenez et al. (2006)

appropriate "microbial formula" tailored for a specific contamination site. Microbes well adapted to a particular environment along with the deficient nutrients, should be considered as an effective strategy for combating oil spills (Raghavan and Vivekanandan 1999). Studies, both lab scale and field, cited in this review clearly indicate that bioaugmentation and/or biostimulation are promising in situ remediation techniques. However, the complexity of the environment makes data prediction based on these studies unreliable so far. Finding competent microbes and conditions for each contaminated site is probably the best solution currently.

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