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# Bioremediation of Mangroves Impacted by Petroleum

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**Abstract** The majority of oil from oceanic oil spills (e.g. the recent accident in the Gulf of Mexico) converges on coastal ecosystems such as mangroves. Microorganisms are directly involved in biogeochemical cycles as key drivers of the degradation of many carbon sources, including petroleum hydrocarbons. When properly understood and managed, microorganisms provide a wide range of ecosystem services, such as bioremediation, and are a promising alternative for the recovery of impacted environments. Previous studies have been conducted with emphasis on developing and selecting strategies for bioremediation of mangroves, mostly in vitro, with few field applications described in the literature. Many factors can affect the success of bioremediation of oil in mangroves, including the presence and activity of the oil-degrading microorganisms in the sediment, availability and concentration of oil and nutrients, salinity, temperature and oil toxicity. More studies are needed to provide efficient bioremediation strategies to be applicable in large areas of mangroves impacted with oil. A major challenge to mangrove bioremediation is defining pollution levels and measuring recuperation of a mangrove. Typically, chemical parameters of pollution levels, such as polycyclic aromatic hydrocarbons (PAHs), are used but are extremely variable in field measurements. Therefore, meaningful mangrove monitoring strategies must be developed. This review will present the state of the art of bioremediation in oil-contaminated mangroves, new data about the use of different mangrove microcosms with and without tide simulation, the main factors that influence the success of bioremediation in mangroves and new prospects for the use of molecular tools to monitor the bioremediation process. We believe that in some environments, such as mangroves, bioremediation may be the most appropriate approach for cleanup. Because of the peculiarities and heterogeneity of these environments, which hinder the use of other physical and chemical analyses, we suggest that measuring plant recuperation should be considered with reduction in polycyclic aromatic hydrocarbons (PAHs). This is a crucial discussion because these key marine environments are threatened with world-wide disappearance. We highlight the need for and suggest new ways to conserve, protect and restore these environments.

**Keywords** Mangrove · Bioremediation · Oil · Microorganisms

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## 1 Mangroves

Mangroves are transitional coastal ecosystems, between marine and terrestrial environments, characteristic of tropical and subtropical regions. In these regions, about 75% of the coastline and nearly 18 million hectares are occupied by mangrove forests (Kathiresan and Qasim 2005). According to Sahoo and Dhal (2009), there are more than 14.5 million hectares of mangrove forests in the Indo-Pacific region (6.9 million), Africa (3.5 million) and the Americas (4.1 million). Brazil, Indonesia and Australia are the countries with the greatest abundance of mangroves (Aksornkoae et al. 1984; Holguin and Bashan 2001).

This ecosystem provides various natural functions of great ecological and economic importance. Some of these functions include being an important nursery for fish, crustaceans, molluscs, reptiles, mammals, bird nesting, a site of carbon and nutrient accumulation, a location of marine biomass renovation and offering protection against coastal erosion (Alongi 2002).

Despite their great ecological and economic importance, mangroves are one of the principle habitats threatened by human action. They are usually located near busy industrial sites (Burns et al. 1993; Li et al. 2007), being considered by some authors as environments under risk of disappearance from the Earth (Lee et al. 2006; Duke et al. 2007). This threat indicates a great need to develop new methods of conservation, protection and recovery for these environments (Duke et al. 2007; Barbier et al. 2008).

## 2 Microorganisms in Mangroves

Mangroves are rich in microbial diversity, and these microbes are fundamental for the maintenance of productivity, conservation and recovery of this ecosystem. Some authors have described that Bacteria, Archaea and Fungi constitute about 91% of the microbial biomass, whereas microalgae and protozoa represent only 7% and 2%, respectively (Alongi 2002). These microorganisms are directly involved in the transformation of nutrients, photosynthesis, nitrogen fixation, methanogenesis, phosphate solubility, sulphate reduction and the production metabolites, such as antibiotics and enzymes (e.g. arylsulphatase, L-glutamines, chitinase, L-asparagines, cellulose, protease and alkaline) (Holguin and Bashan 2001; Das et al. 2006). They are also reservoirs of products of biotechnological interest (e.g. bacteria that produce bioemulsifiers) (Aniszewski et al. 2010).

Although mangroves are rich in organic matter, there is generally a deficiency of nutrients, especially nitrogen and phosphate (Vazquez et al. 2000; Holguin and Bashan 2001). Despite this limitation, mangroves are very productive. This paradox may be explained by a very efficient nutrient recycling system, where the lack of essential nutrients is maintained, and new nutrients are generated by the decomposition of organic matter (Holguin and Bashan 2001). Microorganisms are also responsible for most of the carbon flux in the mangrove sediment, which acts as a carbon drain (Holguin and Bashan 2001). All organic matter in mangroves that is not exported by tides stays in the sediment and is degraded or chemically modified by microorganisms (Kristensen et al. 2008).

Whereas the aerobic microbial community consumes debris that is deposited on the sediment surface, anaerobic microorganisms are fed by debris that is buried by members of the meiofauna and of the macrofauna, by the biomass produced by dead underground roots and the excretion of dissolved organic carbon (Alongi 2002; Kristensen and Alongi 2006; Kristensen et al. 2008). Aerobic microorganisms possess the necessary enzymatic machinery to completely oxidise organic carbon into $CO_2$, but degrading anaerobic processes occur in steps, which involve competition among prokaryotes.

The aerobic degradation of labile materials near the sediment surface of mangroves is generally so fast that the $O_2$ rarely penetrates more than 2 mm into the sediment (Kristensen et al. 1994; Kristensen et al. 2008). Thus, the sediment remains mostly anoxic, except for a network of narrow roots and crab dens that permit the entrance of oxygen (Kristensen et al. 2008). In anoxic conditions, large organic molecules are first divided into simpler compounds by microbial fermentation. These small molecules are then completely oxidised into $CO_2$ by a large variety of anaerobic microorganisms (Kristensen et al. 2008). Aerobic respiration and the anaerobic reduction of sulfate are generally considered the most important respiration processes in mangroves (Alongi 2002; Kristensen et al. 2008). For instance, Lyimo et al. (2009) investigated the anaerobic oxidation of dimethylsulphide and methanethiol in Tanzanian mangrove

sediments and described that this process is dominated by sulfate-reducing bacteria (SRB). Taketani et al. (2010) evaluated the diversity of sulfate-reducing bacteria in a pristine mangrove sediment using denaturing gradient gel electrophoresis (DGGE) and a dissimulatory sulphate reductase (dsrB) gene clone library. The authors described a pronounced effect of depth on SRB diversity. They also indicated a decrease of SRB diversity according to the increase of depth and a dominance of operational taxonomic units related to *Desulfacinum infernum* or to unknown dsrB sequences.

## 3 Mangrove Contamination by Petroleum Hydrocarbons

Recently, anthropogenic practices such as industrial processing, oil spills and incomplete combustion of fossil fuels have caused an accumulation of PAHs in the environment (Chang et al. 2008). PAH contamination is an ongoing environmental concern, and elevated concentrations are commonly encountered in marine and coastal sediment near urban and industrial areas (Yun et al. 2008).

As mentioned earlier, because mangroves are coastal ecosystems, they are among the principal locations where oil spills converge. Different from rocky coasts where the tides assist in washing away contamination, mangroves function as an oil deposit because the circulation of tides favours the deposition of oil in aerial root systems and sediment (Zhu et al. 2001). These environments are also among those with the greatest index of environmental sensitivity to oil spills (Nansingh and Jurawan 1999).

The impact of oil on mangroves, as well as on other ecosystems, is related to the type of pollutant(s), amount spilled, toxicity, the deposition pattern and retention time. All of these factors cause damage to vegetation, which is consequently transferred to other elements that make up the ecosystem, influencing processes and changing local environmental characteristics through chemical and/or physio-chemical modifications. When petroleum and its derivates reach the mangroves, the impact of the physical and toxicological effects may be acute (e.g. defoliation and fauna death) and/or chronic (e.g. reductions in plant reproduction, the survival of seeds the crab population) (NOAA 2002).

Oil spills have caused serious damage to mangroves. For instance, In January 2000, in the Baía de Guanabara, Rio de Janeiro, Brazil, a pipeline rupture caused a 1.3-million-tonne oil spill, contaminating large areas of beaches and affecting mangroves (Brito et al. 2009), especially the north-eastern region of Rio de Janeiro. This event caused strong environmental degradation in forest mangroves near the mouth of the Suruí River. Indeed, there was a mass mortality of forest and the formation of gaps in this area (Soares et al. 2003). However, in 2006, research indicated a process of forest regeneration at the Suruí mangrove (Soares et al. 2006). The impacts from this spill were a combination of acute impacts to water column resources (from diesel) and chronic impacts to intertidal habitats and their associated communities and organisms (from heavy fuel oil) (Michel 2000). Damages to socio-economic resources, including commercial and recreational fisheries, recreational use of beaches, recreational boating and other shoreline recreation, were also reported (Michel 2000).

In 2006, approximately 25 tonnes of oil were spilled in the Port of Gladstone in Queensland, Australia (due to an accident involving an oil tanker), which significantly affected mangroves adjacent to the port (AMSA 2006). Due to the prevailing weather conditions at the time of the spill, some oil was deposited in the intertidal areas, which extended vertically from sediments in the intertidal zone into the mangrove, producing a visible oil line at the high tide mark (Andersen et al. 2008). Some PAH concentrations found at the impacted areas exceeded Australian and New Zealand Sediment Quality Guidelines (ANZECC/ARMCANZ 2000; Andersen et al. 2008). Few to no crab holes were observed in the affected high intertidal area, indicating a low crab density in comparison to reference sites (Andersen et al. 2008). Many other accidents involving oil spills in mangroves have also been reported (Burns and Teal 1979; Wardrop et al. 1987; Corredor et al. 1990; Teal et al. 1992; Burns et al. 1993; Duke et al. 1997; Mille et al. 1998; Burns et al. 2000; Ke et al. 2005; Tam et al. 2005; Yun et al. 2008; Melville et al. 2009; Vane et al. 2009).

In mangrove ecosystems, sediments behave as a reservoir, retaining pollutants. Thus, the toxicity of pollutants is enhanced, affecting the health of the ecosystem. Among these pollutants, petroleum com-

pounds are the most damaging, producing immediate damage to resident organisms (Nansingh and Jurawan 1999; Brito et al. 2009). Mortality and/or damage to plants and animals depends not only on the type, quantity, quality and weathering of oil but also on the prevailing climatic conditions and tides. If the oil spill is not enough to cause tree death, the best management option in mangrove sediments is to only remove the source of contamination, followed by minimal intervention. However, when tree mortality does occur, the degradation of oil in the sediments is severely slowed and mitigation strategies must be applied (Burns and Codi 1998).

Moreover, seasonal variations and biochemical characteristics of the substrates may contribute to the persistence of petroleum within the sediment, increasing the environmental impact (Garrity et al. 1994; Burns and Codi 1998). In such situations, recuperation may take an extremely long time, or the damage may be permanent (Brito et al. 2009).

It is important to draw attention to the fact that different mangrove sediments may present different levels of contamination, depending on the degree of human disturbance to which they are exposed. Tam et al. (2001) evaluated the concentration of PAHs in 20 sediment sampling sites collected in four different mangroves in Hong Kong, finding levels from 356 to 11,098 $ng.g^{-1}$ and described the great heterogeneity in the distribution of petroleum hydrocarbons that may occur in these environments. Maciel-Souza et al. (2006) compared PAH levels found in the Baía de Guanabara (Brazil) mangrove to the sampling sites evaluated by Tam et al. (2001). They observed that the PAH levels in the Brazilian mangrove were between two and six times higher than the highest levels (6.19 $\mu g.g^{-1}$) found in Hong Kong (Tam et al. 2001). In general, PAH levels found in Baía de Guanabara were very high by global comparisons (Maciel-Souza et al. 2006). Thus, one of the great challenges that we encounter when comparing studies of mangrove bioremediation is not only the type and levels of contamination found in each case but also the distribution of the pollutant(s) within the studied mangrove. Thus, the use of bioindicators of oil could provide an efficient tool, in addition to PAH screening, to overcome this challenge

There is not a consensus about acceptable PAH concentrations in coastal environments, but some countries have specific rules for this purpose. For instance, there are no guidelines in Brazilian legislation for environmental quality assessment of coastal sediments for the presence of hydrocarbons. Thus, many authors have used standards (limits) established by other international regulatory agencies (e.g. NOAA and Environment Canada) for 16 priority PAH compounds as references (Long and MacDonald 1998). The Australian and New Zealand sediment quality guidelines (ANZECC/ARMCANZ 2000) are also based on those developed for North American sediments from a large database of matching chemical and biological effects data (Long et al. 1995; Long and MacDonald 1998). However, researchers must use these values with caution because they were established for temperate regions.

Possible destinations for PAHs released into the environment include volatilisation, photo-oxidation, chemical oxidation, bioaccumulation, absorption in soil particles, leaching and microbial degradation (Cerniglia 1992; Yun et al. 2008). It is currently believed that the main processes for efficient PAH removal are microbial transformation and degradation (Gibson et al. 1975; Yun et al. 2008). Some reports suggest that the biodegradation potential of microbial strains isolated from environments contaminated with hydrocarbons is very high because these bacteria may have adapted to the contaminated environment (Wild and Jones 1986; Chaneau et al. 1999; Zhang et al. 2006; Yun et al. 2008).

## 4 Bioremediation

Crude oil is composed of a complex mixture of hydrocarbons, including aliphatic (*n*-alkenes), alicyclic and aromatic hydrocarbons (i.e. PAHs). Some of the most important processes that influence hydrocarbons in the soil are sorption, volatilisation, abiotic transformation (chemical or photochemical) and biotransformation. Sorption and volatilisation do not destroy the contaminants, but only concentrate or transfer them to another medium. Furthermore, abiotic chemical transformations involving organic contaminants are usually slow, and photochemical reactions are insignificant in most environments. However, it is known that bacteria are capable of performing the biotransformation of various contaminants, allowing bioremediation of soils impacted by oil (Korda et al. 1997; Crápez et al. 2002).

The degradation of complex hydrocarbon mixtures in environments is effective in microbial communities with a high associated enzymatic capacity (Alexander 1994). There are many ways to define the reactions that occur during remediation. The term "natural attenuation" may be appropriate to describe all processes used to reduce the level of contaminants that are occurring, including abiotic and biotic processes, where biodegradation is the primary mechanism to reduce the biodegradable contaminants (Nyer 1998). According to Nyer (1998), the term "bioremediation" refers to all biochemical reactions of natural attenuation. This method is considered adequate because if offers low risks to the contaminated sites, and it is an alternative with a favourable cost-benefit ratio for treatment (Korda et al. 1997, Crápez et al. 2002).

The principle of bioremediation is based on the use of microbial populations that possess the ability to modify or decompose certain pollutants. This exploits the genetic diversity and metabolic versatility of the microorganisms for transformation of contaminants into less toxic final products, which are then integrated into natural biogeochemical cycles (Alexander 1994). The action and/or addition of indigenous microorganisms may be used, as well as microorganisms from other genetically modified sites or strains. The main objective of bioremediation is to obtain degradation levels up to the detection limit of the pollutant or below acceptance values established by regulatory agencies. The maximum benefit of this process is the complete mineralisation of compounds, as well as biomass formation (Atlas 1994; Cunha and Leite 2000; Watanabe and Hamamura 2003). The successful biodegradation of petroleum contaminants depends on the presence of specific microorganisms and adequate environmental conditions. In many cases, these microorganisms are already members of the local microflora. Indeed, various bacterial groups present in mangrove sediment are already known for their capacity to degrade hydrocarbons, such as *Pseudomonas, Marinobacter, Alcanivorax, Microbulbifer, Sphingomonas, Micrococcus, Cellulomonas, Dietzia* and *Gordonia* (Brito et al. 2006).

Bioremediation, when possible, is usually applied after the use of physical and chemical methods and natural attenuation and can be a slow process (Cuypers 2001; Zhu et al. 2001; Yang et al. 2009). Its kinetics may be conditioned to various factors (Table 1). Thus, the degradation of oil in mangrove sediment may be much slower than in other locations, taking up to 15 years after large oil spills (NOAA 2002). Typically, when oil enters a mangrove, the cleaning options are physical, chemical and biological, such as the manual removal of accumulated oil, use of absorbent material, pumping of oil trapped in depressions and channels, washing of the sediment and vegetation surfaces using low pressure water jets, use of dispersants and bioremediation (IPIECA 1993; NOAA 2002). However, many of the methods mentioned above become problematic when applied to mangroves, causing side effects that, when added to the impact of oil, may be more harmful to the environment.

In fact, the development and application of bioremediation techniques was given a major boost after the spill of 41 million litres of petroleum from the *Exxon Valdez* in Alaska in 1989. In studies sponsored by the Exxon company on bioremediation from 1993 to 1997, more than 10 million dollars were spent, generating seven patents. In terms of the number of patents, bioremediation was second only to enhanced oil recovery during the first years of implementation. The distribution of patents in specific areas of the oil industry includes 17 patents in soil and water bioremediation and 20 in enhanced oil recovery (Seabra et al. 1999).

The potential application of bioremediation techniques in mangroves contaminated by petroleum has been studied on the Australian continent (Burns et al. 2000; Duke et al. 2000, Ramsay et al. 2000), in Africa (Odokuma and Dickson 2003), China (Ke et al. 2003; Guo et al. 2005; Yu et al. 2005a; Luan et al. 2006) and Brazil (Brito et al. 2009). However, few or no reports are found describing the application of this technique in the field in many countries, until now. Recently, our group evaluated different strategies (Fig. 1) of bioremediation in an oil-contaminated mangrove in Bahia state, Brazil (in situ). Our results demonstrated a better environmental recovery in areas under biostimulation together with bioaugmentation (unpublished results).

## 5 Types of Bioremediation and Mangrove Bioremediation

Bioremediation may be conducted in many ways (Fig. 2) depending on different aspects of the aim, the

**Table 1** The main factors that affect bioremediation in a mangrove

| Variable | Summarising | References |
|---|---|---|
| Contaminants bioavailability | The low bioavailability of PAHs, due to their highly hydrophobic nature and strong tendency to be absorbed by sediment. The application of mobilising agents is an effective way to increase desorption and dissolution of hydrophobic organic contaminants in sediments, and as a result accelerate the biodegradation process. The use of organic solvents or biosurfactants is an alternative to increase the bioavailability of oil and improves the rate of biodegradation | (Alexander 1994; Laha et al. 1995; Jimenez and Bartha 1996; Rebhun et al. 1996; Thibault et al. 1996; Jahan et al. 1997; Luthy et al. 1997; Burns et al. 1999; Conte et al. 2001; Conte et al. 2005; Ke et al. 2005; Sarubbo et al. 2006; Bordoloi and Konwar 2009; Yang et al. 2009) |
| Salinity | Variation in salinity is a predominant factor in mangroves and depends on the behaviour of the tides<br>In some mangroves the most significant factors observed to affect the biodegradation of oil compounds is salinity | (Ward and Brock 1978; Dott et al. 1989, Shiaris 1989; Leahy and Colwell 1990; Trevors et al. 1994; Kastner et al. 1998; Launen et al. 2002; Saponaro et al. 2002; Tam et al. 2002; Olguín et al. 2007; Chen et al. 2008; Wu et al., 2008; Mnif et al. 2009) |
| Temperature | The environmental temperature influences the physical nature and chemical composition of petroleum, the hydrocarbon degradation rate and the composition of the microbial community. According to some authors, the temperature of the sediment may considerably affect degradation rates, where the optimum temperature for biodegradation is around 15–30°C for aerobic processes and 25–35°C for anaerobic processes<br>As the vast majority of mangroves are located in tropical regions, where the average atmospheric temperature in all months of the year is greater than 18°C, the degradation of petroleum is greatly enhanced. Despite this, there is greater breakdown of volatile compounds due to high air temperatures, and the most toxic petroleum components are lost through weathering | (Atlas 1981; Rawe et al. 1993; Balks et al. 2002; Oliveira et al. 2007; Xu et al. 2007; Quan et al. 2009; Yang et al. 2009) |
| Oxygen | Oxygen is generally used as a final electron acceptor in microbial metabolism, and its limitation is a major reason for the reduction of oil biodegradation in mangroves. The importance of oxygen comes from the involvement of oxygenases and molecular oxygen in the main pathways of hydrocarbon degradation. Aerobic production processes possess a higher energetic efficiency per substrate unit and tend to occur much faster<br>Unlike aerobic bacteria, anaerobic bacteria depend on different acceptors to survive. The most common electron acceptors in the natural environment are nitrates, manganese (Mn (IV)), iron (Fe (III)) and sulfate. The quantification of the concentrations of these electron acceptors is very important in the evaluation of anaerobic bacterial activity in the anaerobic biodegradation of PAHs<br>-In the mangroves, the rate of oxygen diffusion in the sediment is reduced due to daily flooding by the tides and does not meet the microbial demand for organic matter oxidation. The decomposition of organic matter then occurs via anaerobic microorganisms that are receptors of alternative electrons to $O_2$. The combination of high levels of organic matter and sulfur in anaerobic conditions, the sources of reactive Fe (through contributions of organic sediments) and the sources of readily available $SO_4^{2-}$, makes the mangrove sediment a propitious environment for the occurrence of bacterial reduction from sulfate to sulfide and the consequent precipitation of Fe in the form of pyrite ($FeS_2$). The | (Atlas 1981; Harbison 1986; Kuznetsov and Ulstrup 1988; Zehnder 1988; Barcelona et al. 1989; Levett 1990; Burland and Edwards 1999; Coates and Anderson 2000; Lyimo et al. 2002; Boopathy 2003; Hwang et al. 2007; Barbier et al. 2008; Li et al. 2009; Tsai et al. 2009) |

**Table 1** (continued)

| Variable | Summarising | References |
|---|---|---|
| | stimulation of anaerobic processes in situ may represent an alternative for the remediation of contaminated areas by organic and inorganic compounds, especially at greater depths | |
| Nutrients | The nutritional state of the ecosystem influences microbial activity and biodegradation. A large limitation for natural degradation of hydrocarbons in the majority of ecosystems is the unbalance C:N:P ratio, caused by the high levels of carbon in the pollutants. Although they constitute a carbon source for the microorganisms, the organic contaminants may not supply additional nutrients, such as nitrogen and phosphorous, in the necessary proportions for the maintenance of metabolic functions. This causes a rapid consumption of the nitrogen and phosphorous available for the microbial hydrocarbonoclastic populations present in the ecosystem, slowing or halting the degradation completely. The most important inorganic nutrients needed for biodegradation are nitrates and phosphates. In mangroves sediments, the export of organic matter and rapid consumption of N compounds leads to a limitation of N | (Vazquez et al. 2000; Holguin and Bashan 2001; Odokuma and Dickson 2003; Kothamasi et al. 2006; Sarubbo et al. 2006; Taketani et al. 2009; Yang et al. 2009) |
| Toxicity | Toxicity generally depends on the concentration and composition of the petroleum contaminant. Acute toxicity usually results from low molecular weight alkanes and aromatic compounds, while chronic toxicity is generally associated with the presence of PAHs. Microorganisms are able to degrade a contaminant if its concentration is below the toxic threshold, but growth and viability are restricted when the concentration of the contaminant exceeds the upper limit. If a contaminated environment is almost lethal to the microorganisms and bioremediation cannot be implemented, special engineering methods must be implemented to extract and dilute the pollutant before initiating bioremediation processes | (Bressler and Gray 2003; Yang et al. 2009) |

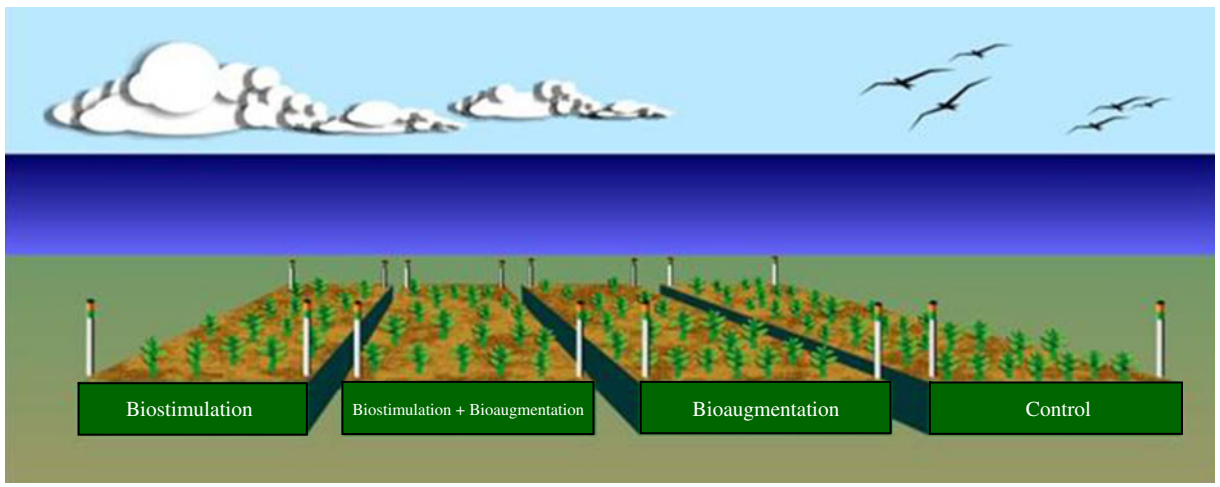


**Fig. 1** Experiment conducted in Brazil evaluating different strategies of bioremediation (biostimulation, bioaugmentation, biostimulation+bioaugmentation and control without treatment) in field (oil-contaminated mangrove in Brazil)

available tools and environmental conditions. For instance, bioaugmentation is a strategy in which the microorganisms that potentially degrade previously selected petroleum hydrocarbons are introduced into the environment (Atlas 1981; Vogel 1996). This method uses indigenous microorganisms, as well as those from other sites or genetically modified strains, in accordance with the laws of each country. The use of microorganisms in the natural biota would be, in principle, more suitable in comparison to those from other sites or genetically modified microorganisms because intensive monitoring would not be necessary (Korda et al. 1997).

The use of an indigenous microbial consortium increased the survival of *Laguncularia racemosa* plants by 35% in mangrove microcosms contaminated with 1% of diesel. Further, total petroleum hydrocarbons (TPHs) analysis indicated that there was a 50% reduction in the levels of TPHs in sediments with the consortium in relation to sediment without the consortium (Peixoto et al. 2009).

Previously, bacteria were isolated in the sediment of a mangrove located in Hong Kong, China, which demonstrated a great capacity for PAH degradation in vitro and could be used to degrade PAH in contaminated sediment (Ramsay et al. 2000). Similarly, Yu et al. (2005a) investigated the biodegradability of PAHs, fluorine (Fl), phenanthrene (Phe) and pyrene (Pyr) by a bacterial consortium enriched with mangrove sediment. The consortium was formed by three bacterial strains: *Rhodococcus* sp., *Acinetobacter* sp. and *Pseudomonas* sp. Inoculation of the enriched consortium caused a small synergistic effect in the degradation of Fl and Phe but only during the first week of growth. According to the authors, further studies should be performed using different consortium combinations and initial inoculum concentrations to elucidate the efficiency of these strains in the bioremediation (by bioaugmentation) of oil-contaminated mangroves (Yu et al. 2005b).

The complexity of the metabolic processes needed to degrade PAHs suggests that no particular microorganism completely degrades petroleum. It is likely that petroleum degradation occurs more efficiently when carried out by complex microbial consortia. Indeed, inclusion of both prokaryotic and eukaryotic species may favour the degradation of a wider range of petroleum contaminants.

Another process, biostimulation, is a bioremediation strategy used to stimulate the ability of the indigenous microorganisms found at the contaminated site to biodegrade pollutants. This stimulation may be caused by the correction of the pH, balancing the nutrients in relation to the carbon load of the organic pollutant, soil aeration, addition of electron acceptors and humidity correction (Burns et al. 2000; Ramsay et al. 2000; Mendelssohn and Lin 2003).

Bioremediation with the application of nutrients involves a variety of techniques and commercial products (e.g. oleophilic formulations and those with slow solubilisation of the components), but usually also incurs high costs (Ramsay et al. 2000; Mendelssohn and Lin 2003). Water-soluble nutrients are represented by a wide variety of agricultural fertilisers, such as nitrogen/phosphorous/potassium (NPK) mixtures with different proportions. The advan-

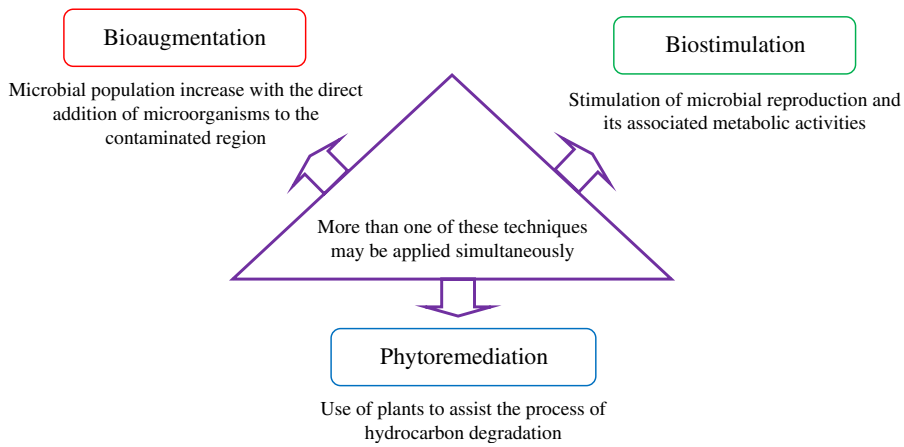


**Fig. 2** Main oil bioremediation strategies definitions

tages of using this type of nutrient include market availability and low cost.

Limitations in the biodegradation of hydrocarbons in a petroleum spill due to a "nutritional imbalance" between the supplied carbon and the nitrogen and phosphorous concentrations needed for microbial growth are found in many studies (Dibble and Bartha 1979; Ramsay et al. 2000; Duke et al. 2000; Mendelssohn and Lin 2003). Rates of nutrient replenishment are usually inadequate to support the rapid degradation of oil, especially if found in high concentrations.

Li et al. (2007) and Burns et al. (1999) highlighted the need to maximise the permanence of the nutrients applied to the contaminated site to reach an efficient and low-cost remediation, especially in environments such as mangrove sediment where the nutrients are often limited. Studies suggest that microbial degradation of oil in mangrove sediment is stimulated by adding inorganic fertilisers (Lee et al. 1993; Odokuma and Dickson 2003; Yu et al. 2005a). However, the effectiveness of this strategy varies from sediment to sediment and contaminant to contaminant (Balba et al. 1998).

The ratio of C/N for biostimulation is highly dependent on the actual situation at the impacted area. The recommended C/N ratio for the bioremediation of hydrocarbons by many authors in the literature varies widely, ranging from 100:1 to 10:1 (Atlas and Bartha 1992; Mosbech 2002), although a working range for C/N of 10:1 is often used (Mosbech 2002). Bioremediation studies conducted by Yuan et al. (2001, 2000) further addressed supplementation with nutrients such as nitrogen and phosphorous, as well as the use of electron acceptors such as oxygen and/or microorganisms.

Phytoremediation is a biotechnological process that utilises the natural physical, chemical and biological processes of plants to remove, degrade, transform or stabilise contaminants found in the soil and groundwater. The capture, transformation, volatilisation and rhizodegradation of hydrocarbons in mangrove sediment are important processes that occur during phytoremediation. Microbial degradation in the rhizosphere (rhizodegradation) may be the main mechanism for cleaning a variety of soils contaminated by petroleum, including mangrove sediment. This occurs because the contaminants, such as PAHs, are highly hydrophobic, and their absorption into the soil reduces their bioavailability for capture by plants and consequently their phytotransformation (Kamath et al. 2004). The success of rhizodegradation depends on the presence of and interaction between specific microorganisms, adequate environmental conditions and the oil availability needed for rhizodegradation to occur.

In a mangrove lacking phosphate, Kothamasi et al. (2006) demonstrated the relationship between five species of the arbuscular mycorrhizal fungus *Glomus* and two *Pseudomonas aeruginosa* strains (phosphate-solubilising bacteria) isolated from the rhizosphere of mangrove plants. The arbuscular mycorrhizal fungus may be considered an intermediary between plants and soil microorganisms, moving carbon and other phytoexudates from the plants to the rhizosphere and transferring nutrients mobilised by the bacteria to the plant (Copley 2000). Sengupta and Chaudhuri (2002) also demonstrated the presence of arbuscular mycorrhizal fungi and their relationship with the mangrove plant community in an estuary of the Ganges River, India. The symbiotic relationship between plants, fungus and bacteria demonstrated by these authors may accelerate the degradation of hydrocarbons in the rhizosphere and optimise the rhizodegradation process.

The main factors that affect bioremediation in a mangrove are described in Table 1.

### 5.1 Case Studies

It is believed that microbial biodegradation is one of the main natural processes used to reduce the concentration of hydrocarbons in contaminated sediment (Hughes et al. 1997). Several groups have reported various studies that focus on the distribution and/or diversity of microorganisms in mangroves, as well as the application of bioremediation techniques in these environments, especially studies performed in vitro.

Ramsay et al. (2000) reported a large number of and a wide diversity of PAH-degrading microorganisms in Australian mangrove sediments. Ke et al. (2003), in a study that used contaminated mangrove microcosms, demonstrated the removal of 90% of pyrane in 6 months. Yu et al. (2005a) reported that the most probable number (MPN) of PAH-degrading microorganisms in the mangrove sediment was about $10^3$ MPN per sediment gramme. Duke et al. (2000) observed an increase in the recovery of plant species

in Australian mangroves affected by oil when they utilised a bioremediation process that included the use of a commercial dispersant, sediment aeration and nutrient biostimulation. Ramsay et al. (2000) also observed that aeration and addition of nutrients significantly stimulates the growth of hydrocarbon-degrading microorganisms in mangrove sediment affected by oil.

The microbial degradation of hydrocarbons has also been demonstrated under anaerobic conditions (Heider et al. 1999; Li et al. 2009). Chang et al. (2008) investigated the effects of different factors on anaerobic degradation of PAHs in mangrove sediment and observed that anaerobic degradation of PAHs is reinforced by the addition of acetate, lactate, pyruvate, sodium chloride, cellulose and iron. Conversely, degradation is inhibited by the addition of humic acid, nonylphenol or heavy metals. The order of the PAH anaerobic degradation rates under these conditions was sulfate-reducing conditions>methanogenic conditions>nitrate-reducing conditions. Anaerobic degradation of PAHs is also inhibited by the addition of molybdate (a selective inhibitor of sulfate-reducing bacteria), BESA (a selective inhibitor of methanogen) and vancomycin (a selective inhibitor of eubacteria) (Lovely and Philips 1988; Distefano et al. 1992), indicating that sulfate-reducing and methanogenic bacteria are involved in the degradation of PAHs. The addition of cellulose and iron increased the anaerobic degradation of PAHs by aerobic respiration stimulation and by acting as an electron donor, respectively (Quantin et al. 2005; Chang et al. 2007). These results also show that the rates of anaerobic phenanthrene degradation were greater than the rates of pyrene degradation (Chang et al. 2008).

Low molecular weight PAHs (two or three condensed aromatic rings) are usually degraded in nature, whereas high molecular weight PAHs (four or more condensed aromatic rings) are considered recalcitrant and often genotoxic (Lin and Cai 2008). Studies of the biodegradation of high molecular weight PAHs in a marine environment, especially in mangrove sediment, have shown promising results, such as the isolation of microorganisms capable of decomposing compounds with four (fluoranthene (Fla) and pyrene) and five (benzopyrene) aromatic rings (Kanaly et al. 2000; Guo et al. 2005; Luo et al. 2005; Yu et al. 2005b; Lin and Cai 2008; Tian et al. 2008b). However, the metabolic pathways by which the marine microorganisms degrade high molecular weight PAHs remain obscure, and bioremediation in marine environments contaminated by high molecular weight PAHs still involves many problems yet to be solved (Lin and Cai 2008).

Some studies have aimed to minimise this problem. Tian et al. (2008a) not only isolated a bacterial consortium that showed an excellent capacity for PAH degradation but also evaluated the impact of the presence of hydrocarbons with different numbers of aromatic rings on the bacterial community of mangroves located at the Jiulong River, in China. Using the DGGE technique, which is detailed below, the authors verified a significant variation in the composition of the bacterial community in accordance with the presence of low molecular weight PAHs (phenanthrene and fluoranthene) or high molecular weight PAHs (pyrene and benzopyrene). It was also shown that the number of dominant species in the presence of pyrene and benzopyrene was much higher than in the presence of phenanthrene and fluoranthene. As a result, it was concluded that (in this case) low molecular weight hydrocarbons were metabolised by pure strains or by simple species compositions. However, the biodegradation of high molecular weight hydrocarbons involved a co-metabolic mechanism, as previously described by Juhasz and Naidu (2000). Therefore, combined efforts of different populations are necessary for the mobilisation and biodegradation of high molecular weight PAHs (Tian et al. 2008b).

Similarly, five stations were established in a mangrove area in Xiamen, China with the objective of isolating a bacteria consortium to degrade benzopyrene (BaP; a high molecular weight hydrocarbon). Five microorganisms were isolated: *Rhodococcus* sp., *Exiguobacterium* sp., *Arthrobacter* sp., *Bacillus* sp. and a new species whose closest relative is *Gordona bronchialis* (84.67% 16S rRNA sequence identity). These microorganisms were inoculated in mineral salt medium (MSM) containing 20 mg $L^{-1}$ of BaP and degraded32.8% of the BaP after 63 days of incubation (Tian et al. 2008a).

Hong et al. (2008) focused on the bioaccumulation and biodegradation of hydrocarbons by diatoms isolated from a mangrove located on the Jiulong River, in China. Two species, *Skeletonema costatum* and *Nitzschia* sp., were exposed to different concentrations of Phe, Fla and a mixture of both compounds.

Both organisms accumulated and were able to degrade the two types of hydrocarbons simultaneously, but *Nitzschia* sp. was more efficient. The values of passive absorption of PHE and Fla by *Nitzschia* sp. were 67.2% and 77.7%, respectively, after 6 h of incubation, whereas the values obtained using *S. costatum* were only 9.9% and 25.0%, respectively. Degradation of Fla by the two algae was slower, indicating that Fla is a more recalcitrant compound for these strains. The microalgae species also showed comparable or greater efficiency in removing a Phe–Fla mixture than Phe or Fla alone, suggesting that the presence of one PAH may stimulate the degradation of another.

Chan et al. (2006) also reported a positive interaction in the degradation of pyrene and two PAHs (phenanthrene and fluoranthene) by *Selenastrum capricornutum*. Similarly, the presence of naphthalene appears to have stimulated the degradation of phenanthrene and pyrene by the *Pseudomonas putida* strain KBM-1 (McNally and Mihelcic 1999). However, inhibitory interactions between two or more PAHs have also been reported in other studies (Bouchez et al. 1995; Stringfellow and Aitken 1995). Dean-Ross et al. (2002) observed that the degradation of pyrene by *Mycobacterium flavescens* is slower when in the presence of fluoranthrene. All of these studies show that when two or more PAHs are present, each of the compounds influences biodegradation of the other PAHs (positively or negatively) depending on the compounds analysed and the strains studied (McNally and Mihelcic 1999; Hong et al. 2008).

Lin and Cai (2008) studied isolates and a bacterial consortium, which degrade high molecular weight PAHs in mangrove sediment in Fujian, China. They also extracted plasmids from strains in the consortium involved in the degradation of pyrene. The consortium, denominated YL, showed degradation capabilities of 92.1%, 87.6%, 92.3% and 95.8% for pyrene, fluoranthene, phenanthrene and fluoeno, respectively, after 21 days of incubation. At the end of the tests, the pH in the media decreased significantly, indicating that intermediate acidic products (e.g. succinic acid, acetic acid, fumaric acid and pyruvic acid) are produced in the degradation process. Two bacterial strains, PY5 (*Bacillus cereus*) and PY6 (*Bacillus megaterium*), were isolated from the consortium and showed pyrene biodegradation rates of 65.8% and 33.7%, respectively. However, only slight changes in pH were detected for both cultures throughout the test, quite different from what has been seen in degradation by the YL consortium, indicating that the type and quantity of intermediate acidic products depend on the strain types.

The plasmids from the bacterial strains of the YL microbial consortium were extracted and used to transform *Escherichia coli* DH5a cells. The cells containing the plasmids degraded 85.7% of the pyrene after 21 days of incubation, which was more than the individual isolates but less than the consortium. However, degradation of pyrene occurred with different kinetics between the YL consortium and the transformed *E. coli* cells. The initial adaptation period of the transformed cells was almost half that observed for the YL consortium. The degradation rate of these cells reached 41.2% after 6 days of incubation, whereas little degradation was observed by the consortium in this same period. Genes or clusters of genes related to the degradation of PAHs have been found chromosomally and on plasmids (Menn et al. 1993; Kiyohara et al. 1994; Cho and Kim 2001; Zhang et al. 2003). For instance, a plasmid encoding the capacity to degrade pyrene has already been identified in a ZL5 bacterial strain that degrades PAHs, which was isolated from soil in an oil field in Liaohe, China (Zhang et al. 2003).

The degradation capacity of the YL consortium and of a single bacteria species, isolated from Huian, China mangrove sediment, was very different from that of microbes isolated from the sediment of other mangroves (Guo et al. 2005; Luo et al. 2005; Yu et al. 2005b), suggesting that mangrove sediments worldwide are sites of innumerable PAH biodegrading microorganisms (Lim et al. 2005).

Tam and Wong (2008) evaluated the effectiveness of various bioremediation techniques (bioaugmentation, phytoremediation, natural attenuation and a combination thereof) for the degradation of PAHs with three, four and five rings in mangrove sediment. The bioaugmentation technique used *Mycobacterium parafortuitum* and *Sphingobium yanoikuyae* isolates. The results show that the two populations were not maintained in the sediment, even with repeated inoculation, suggesting that the isolates did not possess the ability to compete with the indigenous microorganisms and contributed little to the oil degradation. The plant used in the phytoremediation was *Aegiceras corniculatum*, and it was found that the

global PAH losses were comparable to those obtained with bioaugmentation but lower than those obtained by natural attenuation, especially for high molecular weight PAHs. Among the five PAHs tested, low molecular weight PAHs (e.g. Flo and Phe) were easier to remove than the high molecular weight PAHs. In natural attenuation, about 90% of fluorine, 80% of phenothrene, 70% of fluoranthene, 68% of pyrene and 32% of benzopyrene was removed from the mangrove sediment. These results show that the mangrove sediment possesses sufficient indigenous microorganisms capable of naturally correcting the PAH contamination.

Yu et al. (2005a) also compared the effects of natural attenuation, biostimulation and bioaugmentation on the biodegradation of PAHs (Fl, Phe and Pyr) in mangrove sediments. After 4 weeks, natural attenuation was responsible for degrading more than 99% of Fl and Phe but only ~30% of Pyr. Biostimulation with the addition of MSM, a complete solution of nutrients, resulted in the degradation of >97% of all three PAHs, showing that alteration of nutrients may increase the degradation of Pyr. Conversely, bioaugmentation by inoculation of a consortium containing three bacterial species isolated from mangrove sediment (*Rhodococcus* sp., *Acinetobacter* sp. and *Pseudomonas* sp.) showed good potential for PAH degradation in a liquid culture medium. Yu et al. (2005b), reported no effect on the degradation of the three PAHs, which were similar to those of natural attenuation. Thus, natural attenuation appeared to be the most appropriate method to remedy Fl–Phe in this mangrove sediment, whereas biostimulation was more suited for Pyr degradation. This study also shows that, although much (>95%) of the PAH added was absorbed in the sediment at the beginning of the experiment, most PAHs were degraded in 4 weeks. This suggests that the degrading microorganisms efficiently adsorb PAHs.

Similar results were demonstrated in a study performed in a mangrove located on the New Calabar River, Nigeria, where a combination of bioaugmentation (using an indigenous hydrocarbon-degrading bacterial consortium composed of *Bacillus* sp., *Pseudomonas* sp., *Aeromonas* sp., *Micrococcus* sp., *Proteus* sp. and *Arthrobacter* sp.) and biostimulation (using agricultural fertilisers (15:15:15 NPK)) was employed as an option for the recovery of the contaminated environment over 20 weeks. The results of this study show that reduction of hydrocarbon levels can be obtained only through the addition of limiting nutrients. The combination of nutrient addition and the degrading bacterial consortium did not result in a significant increase in biodegradation. Use of nutrients in the form of fertilisers is cheaper than the large scale cultivation of indigenous degrading microbial populations before inoculation in polluted habitats (Lee et al. 1993). In this study, biostimulation was recommended (Odokuma and Dickson 2003).

## 6 Molecular Microbial Ecology and Future Prospects

Despite the sustainable and cost-competitive aspects of bioremediation techniques, the knowledge of factors related to indigenous microbial communities (including diversity and function) at contaminated sites are required for the success of the approach (Desai et al. 2010). This knowledge can be accessed by a classical approach, involving culturing the microorganism(s) in solid or liquid growth medium containing appropriate carbon and electron acceptor sources and a range of other physiological conditions to promote microbial growth. However, general culture conditions impose a selective pressure, preventing the growth of many "uncultivable" microorganisms (Santos et al. 2009).

Recent advancements in molecular tools to study the diversity and function of microbial communities are driving and contributing to a better understanding of microbial ecology, and researchers apply this knowledge to manage and understand biotechnological processes (Santos et al. 2009) such as bioremediation. Currently, the use of molecular tools in microbial ecology is taken as essential, and classic microbiology and molecular microbiology are not longer easily consider as separated sciences (Peixoto et al. 2008).

For instance, DGGE is a molecular fingerprinting technique that provides a pattern or a profile of the genetic diversity in a microbial community, where different bands (generally) correspond to different gene sequences, generating a fingerprint analysis of the target sample (Muyzer et al. 1993). Gomes et al. (2008) explored the diversity of bacterial communities in sediments of urban mangrove forests and suggested that the overall bacterial diversity was not significantly affected by the differing levels of hydrocarbon pollution at different sampling sites.

To determine if the tide influenced the results from microcosm experiments, we compared different approaches to study the microbial community structure in mangrove microcosms. To do this, we constructed three different microcosms; the first one was a 20-L aquarium (40×19 cm) containing 10 L of wet sediment (1.2 Kg/L dry wt.) (Fig. 3a). To mimic tidal action, two pumps were connected to the microcosms, one of which was used to pump artificial seawater (Natural Sea Salt Mix, Oceanic Systems, Texas, USA) into the aquarium (roughly 5 L each time), and the other was used to remove the water after 6 h. This produced an artificial tide that kept the sediment under 5 cm of water for 6 h each time. This procedure was repeated every 6 h. The artificial seawater (20 L) was stored in a 20-L bottle, and 10 L of that water was refreshed every 5 days.

The second system was also a 20-L aquarium (40× 19 cm), but without tide simulation (Fig. 3b). The evaporated water was supplemented every 2 days with distilled water. The water replacement was performed with artificial seawater (Natural Sea Salt Mix, Oceanic Systems, Texas, USA). The third microcosm had smaller proportions (10×8 cm PVC tube), received only 350 g/L dry wt. of sediment and also had no tide simulation (Fig. 3c). A 12:12 h light/dark regime was used to mimic the light period through the use of timers and three 100 W tungsten

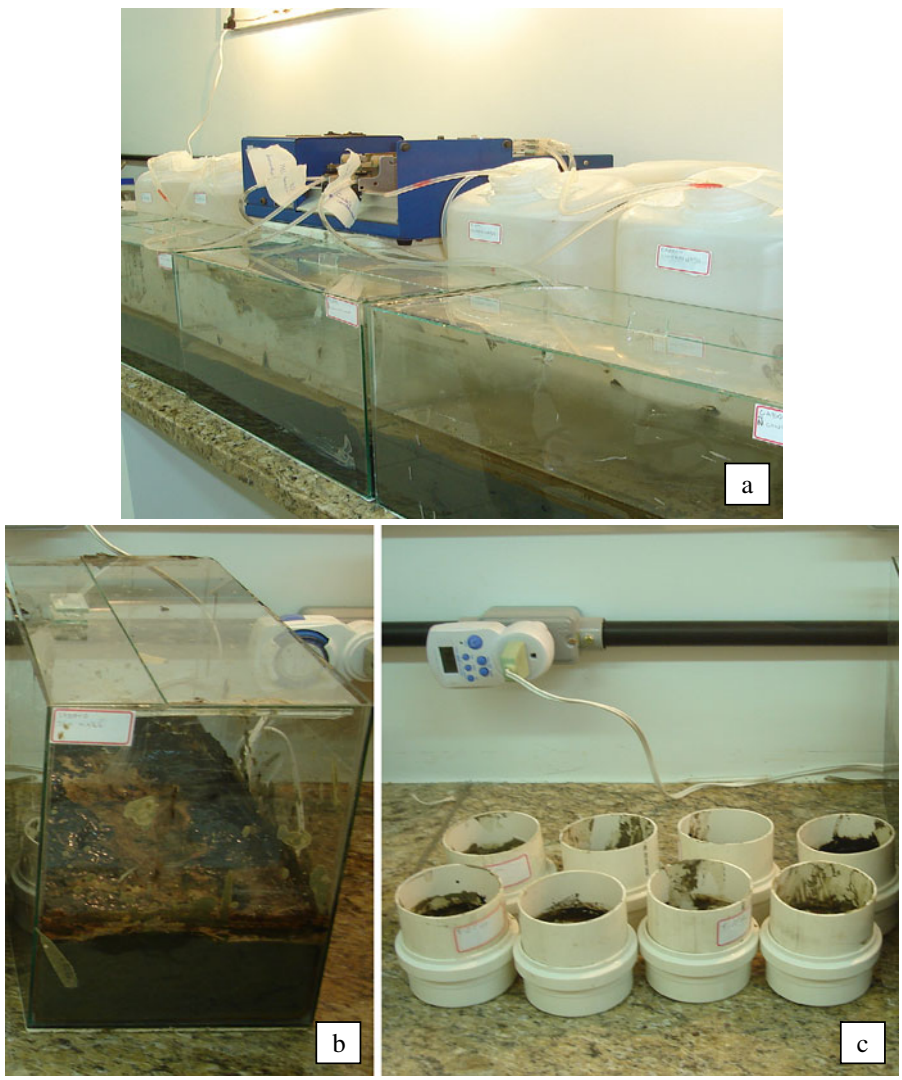


**Fig. 3** The three strategies used in the experiment to evaluate the bacterial community structure in different types of mangrove microcosms; **a** 10-L aquarium with tide simulation; **b** 10-L aquarium without tide simulation; **c** PVC tube microcosm (10×8 cm)

light sources places ~30 cm above all microcosms. After setting up each microcosm, they were left to function for 3 days in order to stabilise and mimic the stratification found in this environment before the onset of the experiment.

The microbial community structure of those microcosms was then compared by molecular tools (polymerase chain reaction (PCR)/DGGE). Our results indicated that the bacterial community structure was very similar between microcosms 1 and 3 (Fig. 4a, b), indicating that the small model without tide was an cost-competitive way to study bacterial diversity in mangroves because it was smaller, easier to reproduce and generated bacterial structure profiles that were similar to those observed in samples from the larger microcosm with tide simulation. Microcosm 2, which was also large but lacked a tide mimic, generated bacterial profiles with some divergence to the other two microcosms, likely due to water accumulation for longer periods of time.

Cloning and sequencing of 16S rDNA have been increasingly used in molecular microbial ecology studies, allowing more comprehensive evaluation of the microbial diversity in environmental samples, including contaminated sites. Taketani et al. (2009) evaluated the relationship between $N_2$ fixation and hydrocarbon degradation in mangrove sediment samples from pristine and contaminated sites, using the microcosm with tide simulation model described in this work (Fig. 3a), with or without added oil, and using clone libraries and Real Time PCR. Their results indicated a greater effect of the addition of hydrocarbons on populations carrying the *nifH* gene in the polluted sample than in the pristine one. In another study (Martin et al. 2006), metagenomic libraries were constructed to understand the ecological and metabolic functions of microbial communities involved in enhanced biological phosphate removal systems.

Metagenomics assesses the total genomic DNA, and thus the metabolic potential, contained within a microbial community. There are two fundamentally different ways to accomplish this: (a) sequence-based and (b) function-based approaches (Warneckea and Hess 2009). These approaches are based on the construction of metagenomic clone libraries, involving

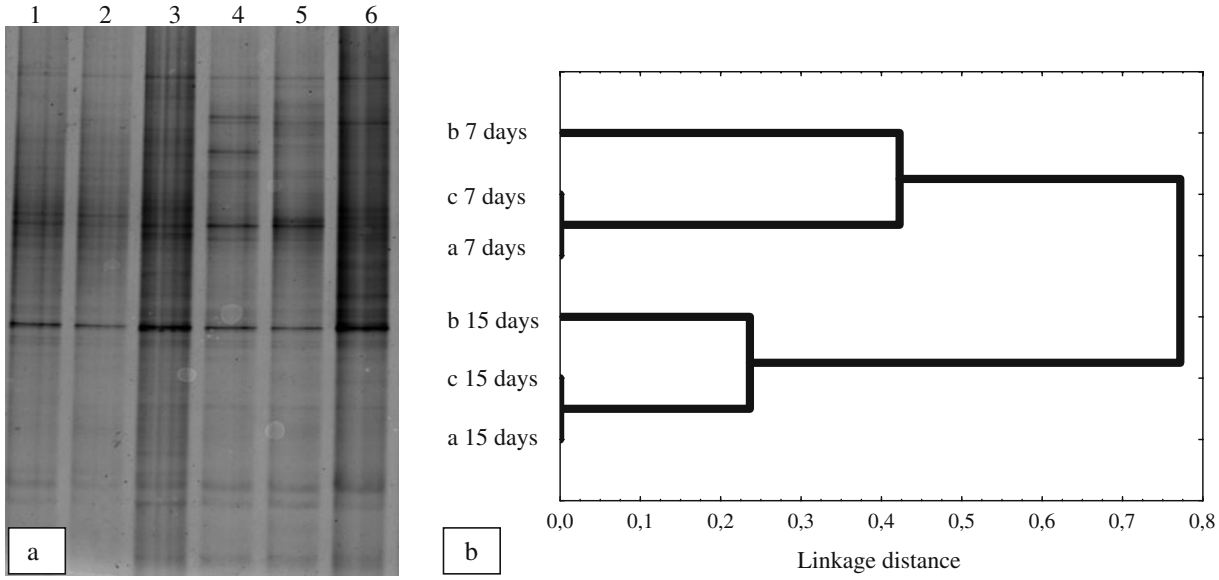


**Fig. 4** **a** Denaturing gradient gel electrophoresis (DGGE) gel of PCR-amplified 16S rRNA gene fragments and stained with SYBR green; *1* 10-L aquarium without tide simulation, 7 days; *2* PVC tube microcosm (10×8 cm), 7 days; *3* 10-L aquarium with tide simulation, 7 days; *4* 10-L aquarium without tide simulation, 15 days; *5* PVC tube microcosm (10×8 cm), 15 days; *6* 10-L aquarium with tide simulation, 15 days. The days represents the time after the microcosm experiment begin. **b** Inferred similarity of bacterial community in 16S rDNA-DGGE profiles from microcosm sediment samples. Dendrograms were constructed from the DGGE gel photo using 1/0 clustering and the UPGMA (Dice Coefficient of Similarity) algorithm; **a** 10-L aquarium with tide simulation; **b** 10-L aquarium without tide simulation; **c** PVC tube microcosm (10×8 cm)

the extraction of environmental DNA, its shearing and subsequent ligation into suitable cloning vectors (Warneckea and Hess 2009). Metatranscriptomics, as well as transcriptomics, emerged as promising methodological approaches for the sequence-based discovery of novel biocatalysts. Using these methods, much smaller sequence space has to be covered because it focuses in on the expressed subset of genes present in the metagenome of a microbial community under certain environmental conditions (Warneckea and Hess 2009). The non-dependence on previous sequence information also represents an advantage of metatranscriptomics approaches.

The development of next-generation sequencing technologies represents a significant step forward for detailed analysis of expression profiles of more complex communities (Warneckea and Hess 2009). To increase the throughput of DNA sequencing, the 454 Life Sciences sequencing platform (454; Branford, CT, USA, Roche) initiated this next-generation movement by pioneering solutions with two limitations: library preparation and the labour-intensive Sanger sequencing method (Mardis 2008; Rothberg and Leamon 2008; Santos et al. 2009; Wall et al. 2009). This next-generation sequencer, presented for the first time by Nyren et al. (1993), is based on the concept of sequencing by synthesis and allows the exhaustive sequencing of DNA fragments. Such an approach will provide more information about microbial diversity in environmental samples and can be applied to evaluate the impact of oil under microbial diversity.

Alternative next-generation technologies have recently become available, aside from those described by Margulies et al. (2005). The first technology to follow 454 was Illumina's (Hayward, CA, USA) Genome Analyzer, developed by Solexa (Cambridge, UK). The second technology is based on the work of Shendure et al. (2005) and is available by Applied Biosystems (Foster City, CA, USA) as the SOLiD system. The system from Helicos Biosciences (Cambridge, MA, USA) does not require PCR amplification of template material, eliminating PCR bias and limitations and enabling true single-molecule sequencing. Such alternative technologies possess different features in comparison with the 454 system (essentially higher throughput but shorter reads) (Wall et al. 2009) that eventually will better suit a particular aim (e.g. metatranscriptomics of complex microbial communities or genetic expression profiling of a given organism in a particular situation). An interesting application would be the metatranscriptomic

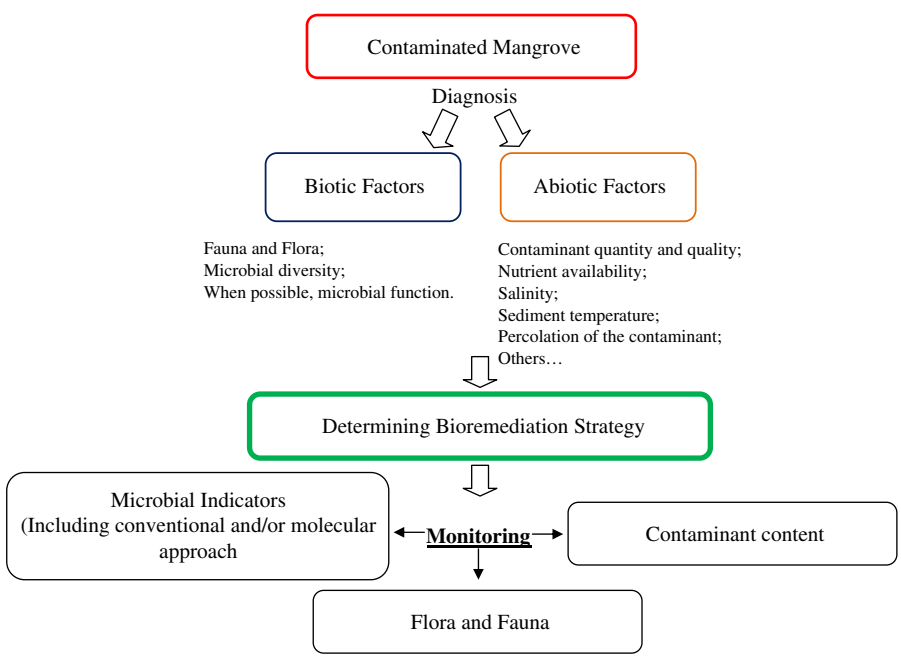


**Fig. 5** Scheme describing important steps of a bioremediation process in an oil-contaminated mangrove

evaluation of mangrove forests focused on the use of experimental studies aimed at revealing the overall community expression profiling in sediment and rhizosphere samples under normal and oil-polluted circumstances. Such a survey would provide valuable information about prevailing functional genes expressed in oil-polluted and non-contaminated sites.

DNA microarrays can be also a powerful tool in microbial ecology, although there are known limitations when compared to metatranscriptomics (e.g. microarrays are always dependent on previously known sequence information, present problems with interpretation of signal intensities etc.). Such a technique can be classified into different types (Bae and Park, 2006): to evaluate microbial community structures (PhyloChips) or to evaluate function (functional gene arrays) (He et al. 2007) and whole genome arrays (Desai et al. 2010). The microarray technique is based on hybridisation of the DNA target, including community genomes, to the array probes, followed by measurement of fluorescence signals. (Desai et al. 2010). In a valuable approach to compare two distinct field bioremediation experiments in Arctic stations, Yergeau et al. (2009) assessed the bacterial community structure and function using microarrays targeting the 16S rRNA genes of bacteria found in cold environments and hydrocarbon degradation genes, as well as quantitative reverse transcriptase PCR targeting key functional genes. They reported divergences in soil microbiology and decontamination rates between sampling sites and indicated relationships between key gene expression and bioremediation treatments, demonstrating the value of monitoring microbial diversity and function during bioremediation using molecular techniques.

Bioremediation strategies can be improved by a greater knowledge of microbial biology, diversity and function, and new molecular technologies can support this development. Genomics, for instance, provided complete genome sequence data for several microorganisms that are significant for bioremediation, such as *Pseudomonas*, *Shewanella*, *Sphingomonas*, *Arthrobacter* etc. (Desai et al. 2010). However, additional studies of microbial molecular ecology are necessary to generate information about oil contamination effects on microbial communities, as well as about the effects of bioremediation techniques on microbial diversity.

## 7 Final Considerations

Many authors have concentrated their efforts on understanding the impacts caused by the presence of oil on microbial communities in the sediment and rhizosphere of mangrove plants. These studies have produced a better understanding of the variables that influence oil degradation in these environments, generating data for optimisation of the bioremediation process in mangroves impacted by oil.

Before applying a bioremediation strategy, however, it is important to diagnose each contaminated environment individually. One must determine the biological diversity at the site and the abiotic factors that influence the biodegradation of the contaminant, such as the quantity and quality of the contaminant found at the site, nutrient availability, salinity and temperature of the sediment, among others (Fig. 5).

After the initial evaluation, bioremediation strategies may be determined, for example, to accelerate the natural attenuation process of that particular mangrove. This anticipation of the mangrove recovery may result (and should result) in faster re-colonisation and/or visitation of animals and the survival and growth of plants, which is often crucial to the recovery of the contaminated site prior to its complete deterioration.

Finally, our major concern is determining how to establish the acceptable levels of PAH contamination in mangroves and, especially, how to efficiently monitor such values in such a heterogenic environment. Our suggestion is that the entire environment must be evaluated to determine what is acceptable and not, including PAH levels, plant health and survival, the presence of animals and molecular microbial profiles. This multi-parameter evaluation should be the gold standard for mangrove bioremediation and monitoring.

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