

Review

# In Situ Aerobic Bioremediation of Sediments Polluted with Petroleum Hydrocarbons: A Critical Review

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**Abstract:** Oil pollution has been a worldwide concern especially in environments where treatment is quite difficult to apply. Marine polluted sediments, in particular, constitute one of the most recalcitrant environments for bioremediation and are often the final repository of petroleum contaminants, as a result of runoff and deposition. Aerobic hydrocarbon degraders present in the sediments are tackling the pollution under oxygen-limited or oxygen-depleted conditions. Research has focused on new ways to enhance bioremediation under anoxic conditions, however aerobic bioremediation is faster, and hence more effort should be made to sustain oxygen concentration levels. In this review, the different bioremediation techniques used for the decontamination of marine sediments are briefly discussed, and focus is primarily given to the different oxygenation methods used for enhancing aerobic bioremediation and the aeration methods that are suitable for in situ application, as well as state of the art technologies that make in situ aeration an appealing approach. Based on the technologies analyzed, suggestions are made for sediment bioremediation techniques in different marine environments.

**Keywords:** sediments; bioremediation; in situ oxygenation; fine bubbles; marine environment



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## 1. Introduction

With 5.72 million tonnes of oil spilled as a result of oil shipping accidents between 1970 and 2015 [1], and over 1.45 million tonnes of oil spilled due to marine oil rig accidents [2,3], oil spills constitute a major environmental management problem, adversely affecting the marine ecosystem and, consequently, human life. The marine environment has been the receiver of large quantities of hydrocarbon pollutants that ultimately end up on the sea floor and get absorbed by the sediments [4]. To date, different response strategies have evolved to treat petroleum-contaminated environments [5,6], but sediment contamination, which constitutes a serious environmental concern for the deterioration of the ecosystem, is often overlooked.

Bioremediation is a process that utilizes the natural capacity of microorganisms to degrade or detoxify hazardous waste [7]. Engineered bioremediation modifies the environmental conditions (physical, chemical, biochemical, or microbiological) to enhance the degradation capacity of the native microorganisms [8]. It is an ecofriendly approach for restoring contaminated ecosystems without causing additional damage. Sediment bioremediation techniques have been applied over the years in different aquatic environments; however, they are limited to shallow applications due to implementation difficulties in deeper environments. As research progresses, more and more techniques have been developed to overcome these difficulties [9]. The importance of engineered bioremediation

and natural attenuation came to the foreground after the Exxon Valdez oil spill, where a storm washed most of the oil to the shorelines [10]. The effectiveness of bioremediation is interchangeably linked to the capacity of the indigenous microorganisms to degrade and detoxify chemical contaminants. Field research after the Deepwater Horizon accident showed that indigenous microbial communities play a pivotal role in oil spill remediation [11]. Exploiting these hydrocarbon-degrading microbes for bioremediation purposes is of great importance. It has been shown that, in the presence of adequate oxygen, biodegradation rates of hydrocarbons are very fast, yet under anaerobic conditions, which is the case for most sediments, bioremediation can be an extremely slow process [12]. Hence, oxygen availability is fundamental for fast hydrocarbon biodegradation and can shape the bioremediation time. This realization raises the need for development of efficient aeration and oxygenation techniques for the sediments to promote and enhance aerobic bioremediation.

Bioremediation techniques are distinguished in two broad categories based on the site of application [5–7]. *Ex situ* techniques involve the excavation of polluted sediments from the site of contamination and their subsequent transfer to another site for treatment. The so-called dredging of sediments comes with a number of considerations since it can be the cause of deeper penetration of the contaminants into the sediments and it can also cause the release of the contaminants to the water column, negatively affecting the surrounding ecosystem. *In situ* bioremediation, on the other hand, is a highly promising and cost-effective technology for the sustainable remediation of contaminated sites. *In situ* techniques can be categorized into engineered and intrinsic bioremediation. Intrinsic bioremediation relies solely on the microorganisms to return the environment to its original state prior to contamination, while engineered bioremediation is either based on stimulating the autochthonous microorganisms for faster biodegradation of the pollutants, or on the addition of allochthonous microorganisms specifically targeted to degrade petroleum hydrocarbons with ease [13].

Besides large-scale catastrophic events, lesser but long-lasting pollution hydrocarbon pollution, such as that occurring in ports, has been overlooked. Harbors and marinas are hot spots of contamination dominated by polycyclic aromatic hydrocarbons (PAHs) produced by leaks (petrogenic) or combustion processes (pyrogenic), with the latter being less bioavailable and thus posing additional challenges for bioremediation [14]. While better monitored due to urban area proximity, this type of pollution has been taken more or less for granted. Nevertheless, in the context of Green Ports as part of the global environmental agenda and the sustainable development goals, especially those for life below water and future-proofing infrastructure, ecosystem restoration and environmental sustainability in the port environment has become more relevant. *In situ* aerobic bioremediation techniques may have additional advantages here as they require limited space, cause minimum disturbance, and can lead to faster removal of recalcitrant compounds.

## 2. Engineered Bioremediation

### 2.1. Bioaugmentation

Bioaugmentation involves the implementation of cultured microbial populations into a contaminated zone to promote biodegradation of the contaminants. These microbial populations include pure bacterial strains, consortia, genetically engineered bacteria, and genes transferred to the indigenous microorganisms [15]. The application of microorganisms preadapted to the contamination seems very promising. However, the scientific community is torn between the actual effects of bioaugmentation. The introduction of allochthonous microorganisms in field studies for the bioremediation of oil contaminated sediments has shown little enhancement of biodegradation in some cases [16–18], while other research studies support the technique's effectiveness on hydrocarbon bioremediation [19–23]. Allochthonous microorganisms might be equipped to battle hydrocarbon pollution but there are several factors to be considered for an effective application. Environmental conditions and even competition with the indigenous microorganisms could lead in limited action

of the introduced microbes [17,24]. Autochthonous bioaugmentation (ABA) has been developed to overcome such implications [25]. Examining the indigenous microorganisms, classified for hydrocarbon degradation, on their native environment, could be an effective alternative to combat oil pollution. In a bioremediation study simulating an oil spill event, it was shown that the application of ABA coupled with biostimulation is a promising bioremediation strategy [26].

## 2.2. Biostimulation

Biostimulation of the indigenous microbiome to battle hydrocarbon pollution is a widely used bioremediation technique for aerobic sites, but its use is hindered in oxygen-depleted sediments [27]. There are several factors that can affect the rate of hydrocarbon degradation including environmental factors, the type of sediments, the autochthonous microorganisms, and the type of hydrocarbons and the extent of the pollution. Among the environmental factors are nutrient and oxygen availability, hydrocarbons bioavailability, pH, and temperature [28,29]. Biostimulation is employed to overcome some of these limitations and prolong the biodegradation efficiency of the microorganisms. Besides oxygen and nutrient addition as biostimulation strategies, other techniques can fall into this category, such as the use of chemical dispersants and surfactants to enhance bioavailability of hydrocarbons to the microbes [30], and phytoremediation, as a means of soil aeration in some cases [31].

### 2.2.1. Nutrients

Nutrients are available in most aquatic systems, however under the stress of petroleum contamination, nutrients can be depleted after a while due to the increased metabolic activity of the microorganisms in their efforts to degrade the contaminants. In most highly hydrocarbon contaminated coastal systems, nutrient availability is the limiting factor for biodegradation [5,32]. The most commonly employed elements in hydrocarbon degradation are carbon, nitrogen, and phosphorous. Typical requirements of these elements for the metabolic activities of the microorganisms are in a C:N:P ratio of 100:10:1 with some studies reporting optimum results for crude oil bioremediation of ratios ~70:3:0.6 [33] and 8:1:0.07 [34]. Nutrients are usually introduced in the form of inorganic salts, urea, anhydrous ammonia, and fertilizers [35,36]. However, direct application can lead to unnecessary losses into the environment due to wash out such as in the case of open seas and intertidal environments. Efforts have been made to develop nutrient delivery systems capable of overcoming these implications. Slow-release fertilizers and oleophilic fertilizers present a better distribution of nutrients into the contaminated system. They were first introduced for bioremediation purposes after the Exxon Valdez oil spill and have been proven an effective biostimulation strategy ever since in a number of in situ applications for sediment bioremediation [37–42].

### 2.2.2. Oxygen

Nutrient addition is not expected to present any significant enhancement in oil biodegradation rates if oxygen is depleted [43,44]. Oxygen is the main electron acceptor in aerobic bioremediation, and, if not present in adequate concentrations, can limit the biodegradation potential of aerobic microorganisms. In the absence of oxygen, anaerobic bioremediation, which has been proven to be a very slow process compared to aerobic bioremediation [12,45], becomes the dominant process. Oxygen demand for sustaining and promoting aerobic bioremediation can be significantly high. In the case of hydrocarbon pollutants, different studies estimate different stoichiometric ratios of oxygen required for complete degradation of the contaminants: 3 M O<sub>2</sub> for 1 mole of hydrocarbon [46], 3 kg of oxygen for every kg of petroleum product degraded [47], 8.6 mole oxygen for every mole of diesel fuel degraded [48], and 0.5–1 g oxygen/g hydrocarbon [49]. However, for the variable and dynamic conditions of the marine environment, these requirements can only be roughly estimated and are quite difficult to meet. Different techniques have been

developed for the supplement of oxygen in contaminated environments and are further discussed in Section 3.3.

### 2.2.3. Dispersion of Oil

Chemical dispersants and surfactants consist of a common bioremediation strategy, but their use is controversial and their actual effect on microorganisms has been questioned. Dispersants are chemicals that break down oil into fine droplets to make it more bioavailable for microbial degradation. When applied on surface oil slicks, dispersed oil is mixed with water due to wave activity and enters the water column, becoming prone to microbial degradation. The primary purpose of dispersants use, on oil spills that occur in the sea, is to not reach sensitive shoreline habitats, like marshes and mangroves [50]. Dispersants constitute one of the first response actions in the occurrence of an oil spill, but are applied after careful consideration, since they can have a toxic effect on living organisms. Dispersant toxicity should also be taken into consideration when applied for sediment bioremediation, as it could inhibit microbial metabolic activities. Ferguson et al. [51] examined the dispersant activity on oil biodegradation in subarctic deep-sea sediments, and their results showed that dispersant addition led to significant increase in the rate of degradation at sediments collected from 1000 m deep, but had no effect at 500 m, thus indicating an ambiguous effect of the dispersants and a further need for research studies.

Biosurfactants have gained popularity in the recent years over the respective synthetic surfactants. Biosurfactants are amphiphilic molecules of microbial origin with surface active properties that reduce the surface tension and facilitate the contact between microorganism and pollutants [52]. They present all the benefits of the chemical surfactants with the addition that they are not toxic to microorganisms and provide higher biodegradability [52]. Biosurfactants can also maintain their activity under extreme conditions of pH, salinity, and temperature [53,54]. Their use for oil bioremediation purposes has been highlighted in numerous studies [55–57]. Nevertheless, industrialized biosurfactant production remains a financially unviable option compared to chemical surfactants [58].

### 2.2.4. Phytoremediation

Phytoremediation is considered a biostimulation strategy for the degradation of hydrocarbon pollutants that exploits the natural growth processes of plants and their associated microorganisms. Phytoremediation is an attractive potential for petroleum hydrocarbon cleanup in terrestrial environments [59,60] but has also gained attention for the decontamination of sediments in sensitive aquatic environments such as salt marshes and mangroves [61,62]. Restoration of such ecosystems is very tricky and challenging [63,64]. Salt marshes and mangroves are nursery habitats for a plethora of marine organisms and plants. They serve as a buffer between land and sea, filtering nutrients and absorbing toxic compounds, and providing a shield to coastal areas from natural phenomena and erosion. Phytoremediation is primarily based on the inherent ability of wetland plant species to aerate the soil rhizospheres for the stimulation of aerobic oil biodegradation. Wetland plants might accumulate quantities of oil and release exudates and enzymes that enhance microbial activity. Phytoremediation alone has been shown to significantly enhance microbial degradation of oil in mangrove sediments in regard to natural attenuation [62]. However, when combined with fertilizers for nutrient supplement, phytoremediation may lead to superior results [65].

### 2.3. Intrinsic Bioremediation

Intrinsic bioremediation, also called natural attenuation, solely relies on the microbial degradation potential [66]. Indigenous microorganisms have adapted to their environment and, if previously exposed to petroleum contamination, might be equipped with defense mechanisms and respond immediately to an oil spill event. Natural attenuation is a cost-effective technique since no intervention is required; however, monitoring is required

to determine the sustainability of the process. Prior to application, risk assessments should be conducted to eliminate the risk of human exposure to contaminants. Intrinsic bioremediation is not a very attractive choice for the bioremediation of sediments since it is a very slow process, especially under the prevailing anaerobic conditions of the deeper layers.

### 3. Aerobic Bioremediation

In general, the applicability and efficiency of bioremediation depend on various factors. The primary criteria are based on the geographical and geological characteristics of the contaminated site (e.g., offshore locations, type of sediment), the nature and amount of contaminants, environmental constraints that may apply, and cost of application [67,68]. Biodegradation rates are also linked to environmental conditions and microbial community characteristics. Environmental conditions such as pH, temperature, availability of nutrients and oxygen, high hydrostatic pressures and the bioavailability of contaminants can significantly shape the bioremediation efficiency [28,31].

Sediment bioremediation is often hindered by some of these environmental conditions such as the prevailing high hydrostatic pressures (e.g., deep-sea polluted sediments) and anoxia. Even though bioelectrochemical approaches have been evolved to enhance anaerobic degradation of contaminants by providing different electron acceptors (microbial fuel cells [69–71], snorkels [72–75], cable bacteria [76–80]), they still fail to meet the biodegradation rates achieved under aerobic conditions. Hence, in this work we focus on aerobic bioremediation applications on shallow sediments.

#### 3.1. Oxygen Profile in Marine Sediments

The oxygen profile in marine sediments strongly depends on the aquatic zone examined. Open seas offer a bigger span in the oxic zone, up to a few decimeters in depth [81], while on continental margins, oxygen penetration depth ranges from mm up to a few centimeters [82]. Coastal sediments on the other hand, typically present oxygen penetration depths of a few millimeters unless they are comprised of very permeable sandy sediments [81]. In general, oxygen penetration can be deeper in areas with low sedimentary respiration [83]. Oxygen concentration in the sediments is dependent upon a lot of factors. Temperature, depth, permeability of the sediments, tidal and wave forces, extent of pollution, redox compounds, and even salinity [84] can affect the dissolved oxygen (DO) concentrations. For instance, for the past half century the increasing temperature of the oceans has led in ~2% DO decline in the seawater [85]. DO has also been shown to diffuse only within the top few millimeters in the sediments even if the overlying waters are saturated with oxygen [86].

Oxygen is the most important electron acceptor for biodegradation processes [87]. Microorganisms under aerobic conditions can easily degrade various pollutants that accumulate on the sediments and pose a threat to sensitive ecosystems. For instance, the presence of hydrocarbon pollution in marine sediments can lead to a quick consumption of oxygen due to the continuous uptake from microorganisms to degrade the contaminants. Oxygen consumption leads to the alternation of aerobic conditions to anoxic and even anaerobic with subsequent alternation in the microbial populations. Besides the oxygen uptake from the microorganisms, oxygen depletion can occur due to abiotic reactions in which oxygen takes place. Therefore, knowing the oxygen profile of sediments that face petroleum contamination is essential to adjust the bioremediation strategies accordingly.

#### 3.2. Microbial Community and Metabolism Affected by Shifts in Sediments' Oxygenation

The indigenous microbial population of marine sediments is interchangeably linked to the presence of oxygen. Shifts in sediment oxygenation result in shifts in microbial communities as different metabolic pathways prevail. In the oxic layer, aerobic biodegradation is promoted. Since the oxic layer is typically very thin, ranging from mm to cm, but microbial activities are known to be present even at great depths into the sediments,

anaerobic metabolism is dominant. Nevertheless, oxygen deficiency in sediments can lead in changes in the microbial populations that could ultimately harm marine ecosystems and lead in alterations in the biogeochemical cycles [88]. A comprehensive study by Broman et al. [89] examined the changes in microbial populations and encoded genes for metabolic processes inflicted by shifts in oxygen levels in coastal oxic, intermediate, and anoxic zones. Their results supported that oxygenation of the sediments had a high impact on microbial communities. From experiments in the oxic-anoxic zone, they showed that anaerobic microorganisms are more likely to adapt in the presence of oxygen rather than the aerobic ones under anoxic conditions. Oxygenating anoxic sediments is likely to limit the release of produced methane to the atmosphere and re-oxygenating anoxic sediments can lead to an approximate 2.5 months of restoration of coastal dead zones [90], indicating that oxygenation is in fact an effective remediation strategy for marine sediments.

### 3.3. Oxygen Amendments

Providing oxygen is essential for bioremediation processes and can lead to a faster biodegradation of petroleum hydrocarbons, even the persistent heavier and aromatic compounds. An adequate supply of oxygen is of great importance to sustain the aerobic biodegradation of the indigenous microbiome. A number of technologies have been developed to provide oxygen for the enhancement of aerobic bioremediation. The most common techniques are presented below.

#### 3.3.1. Oxygen Releasing Compounds

Oxygen releasing compounds are engineered chemical formulations, mainly designed to enhance in situ aerobic bioremediation of petroleum hydrocarbons in various aquatic environments including marine environments. However, their use can be expanded to sediment applications, when dispersed in a powdered form for the treatment of contaminants. The original Oxygen Releasing Compound (ORC<sup>®</sup>) is a patented phosphate-intercalated magnesium peroxide [91], which when in contact with water, produces a controlled-release of oxygen (10% *w/w* of molecular oxygen) [8] with up to a year of activity on a single application. Both the ORC and the produced hydroxide from the water reaction are environmentally benign and can be safely ingested, making this method suitable for direct application in sensitive ecosystems without a particular need for monitoring [92]. Their use is well established since they have been extensively tested over the past 27 years since their first appearance. It is proven that ORCs can accelerate aerobic biodegradation 10 to 100 times compared to the intrinsic rates of biodegradation [91], and accompanied by their long-lasting application, they are considered a highly cost-effective method for enhancing aerobic bioremediation. However, since oxygen can be incorporated into abiotic reactions as well, significant amounts could be consumed rapidly in undesirable reactions. Other types of peroxides such as calcium peroxide have also been employed for the release of oxygen in contaminated sediments [93]. Newly developed technologies of oxygen slow releasing materials (OSRM) [94] have been implemented for the bioremediation of hydrocarbon polluted sites and, by altering the concentration of the embedding medium, oxygen-releasing rates could be adjusted to the requirements of the aquatic environment. However, magnesium peroxide is the usual preference due to its low solubility, and the ability to release oxygen for a longer period [95]. ORCs have also been tested in research studies for the bioremediation of marine sediments as a biostimulation strategy [96]; however, their application in the marine environment has not been addressed in detail [63].

#### 3.3.2. Pure Oxygen Injection

Pure oxygen has been widely used for the ex situ biodegradation of organic pollutants, but its in situ application is also feasible. Pure oxygen injection is another technique designed to promote aerobic bioremediation. Oxygen is introduced in its vapor phase (approximately 95% oxygen) into the saturated zone via vertical injection wells, specifically placed to meet the needs of the contaminated area. Oxygen is stored in a bulk tank, concen-

trated in a liquid form at  $\sim -155^\circ\text{C}$ , and then passed through an ambient air vaporizer that adjusts the flowrate. Vapor oxygen is then injected to the site through spargers or other types of diffusers [97]. Compared to air injection, where atmospheric air is introduced to provide oxygen, pure oxygen injection could cause a four to fivefold increase in the dissolved oxygen concentration. To achieve high dissolved oxygen concentrations, oxygen is sparged at a low flow rate to optimize the contact time between oxygen and the contaminated zone which could be further increased by oxygen entrapment in the sediments. Low flow rates also reduce the risk of producing volatile organic vapors and migrating of the organic pollutants [8]. Field demonstrations of the method in polluted river sediments have proven the technique's effectiveness in stimulating the aerobic biodegradation rates without affecting the overlying water quality [98].

### 3.3.3. Hydrogen Peroxide Infiltration

Hydrogen peroxide infiltration is a controversial method regarding its true impact on the environment. However, its application is considered to provide one of the highest levels of available oxygen to the contaminated area: half of the amount introduced to the site is stoichiometrically expected to provide molecular oxygen ( $\text{H}_2\text{O}_2 \rightarrow \frac{1}{2} \text{O}_2 + \text{H}_2\text{O}$ ). Hydrogen peroxide is introduced to shallow contaminated sediments by a system of pipes or sprinklers, but for deeper applications, injection wells are used [99]. When in contact with water,  $\text{H}_2\text{O}_2$  decomposes rapidly, making it difficult to treat target zones and qualifying it as a cost-intensive process. Hydrogen peroxide may also be involved in non-beneficial reactions, both biotic and abiotic, resulting in significant losses in oxygen production [100,101]. Efforts have been made to stabilize  $\text{H}_2\text{O}_2$  to prolong its decomposition as an oxygen provider but with little to no success so far [102]. In the presence of an iron catalyst, a concentrated form of hydrogen peroxide is used as a chemical oxidant (Fenton's reagent) to produce highly toxic hydroxyl free radicals [103,104]. Microorganisms are not capable of withstanding large amounts of hydrogen peroxide. A typical concentration limit is 100–200 ppm [8] after which  $\text{H}_2\text{O}_2$  becomes cytotoxic. Hence, the in situ use of hydrogen peroxide should be determined after a number of factors are taken into consideration such as the soil geochemistry, the native biota, and the type of contaminants [105]. The technique has been proven to enhance DO in lakes with positive results in enhancing organic matter removal from the sediments [106]; however, a field study on the bioremediation of organically polluted marine sediments, presented by Thomas et al. [107], showed no enhancement on the degradation of organic pollutants.

### 3.3.4. Ozone Injection

Ozonation transforms residual organics into available biodegradable products, including organic acids, and releases nitrate and reactive phosphate; hence, it should be able to accelerate the rate of total petroleum hydrocarbons (TPH) reduction during bioremediation, and reduce the need for external addition of nutrients. Thus, ozonation could enhance bioremediation by the removal of heavy petroleum hydrocarbons in the field [108]. Ozone injection, as well as hydrogen peroxide infiltration serves a dual application: ozone is used as a chemical oxidation treatment, while at the same time provides a substantial source of oxygen for the bioremediation processes. As a chemical oxidant, ozone can inhibit microbial activity, but this effect seems to be temporary, since the surviving microorganisms from ozone application are sufficient to sustain bioremediation [109]. Hydrogen peroxide and ozone are two of the most commonly employed chemical oxidants [110], but the in situ application for the bioremediation of organic polluted sediments is often inhibited by the unknown environmental side-effects and the difficulty of direct application on the sediments [111]. Ozone is added in dissolved or gaseous form and can diffuse into soil aggregates promoting aerobic bioremediation. It is 10 times more soluble in water than pure oxygen and when applied in the subsurface, ozone reacts with the organic pollutants and decomposes rapidly into oxygen. So far, no information is available concerning the proper dose of ozone that gives minimal mineralization [108]. A typical application involves

injection or sparging of ozone gas at a 5% concentration in the contaminated area [109]. Due to the production of gases, soil vapor extraction wells might also be required.

#### 4. Sediment Aeration

Besides the oxygen amendments, aeration techniques can be used to promote aerobic bioremediation. Sediment aeration is an essential process for enhancing the aerobic biodegradation of hydrocarbon pollutants that end up on the sediments of aquatic environments. Different aeration systems have been developed and can be applied depending on the type of aquatic environment (e.g., lakes, rivers, ponds, shorelines, ports, etc.). Especially in the case of marine environments, where sediments are the ultimate receiver of contaminants and anoxic conditions prevail and inhibit biodegradation processes, it is of great importance to generate new knowledge and solutions for a sustainable sediment aeration system. Sand is a high permeability sediment, hence the use of a form of aeration to provide oxygen could be practical. The most common sediment aeration techniques, including newly developed promising systems, are presented here.

##### 4.1. Aeration Systems

###### 4.1.1. Tilling

Tilling, which is also called mixing or aeration, is a physical method that disturbs the oiled sediment layer and increases the penetration depth of oxygen and nutrient supplements [112]. The goal of this method is to accelerate the natural oil removal process, by exposing the oiled sediments to weathering processes. Additionally, by mixing the sediments, oil is subjected to natural physical degradation, while nutrients, if applied, and oxygen can penetrate deeper into the sediments thus enhancing microbial degradation processes. Field demonstrations of the method on shallow sediments, however, show no significant effect of tilling on biodegradation rates [39,113,114]. The application of tilling is inhibited in sensitive ecosystems, such as salt marshes and wetlands, due to its destructive impact on vegetative growth [115].

###### 4.1.2. Biosparging-Forced Aeration

Biosparging is a technique designed to introduce air for stimulation of the aerobic microbial community. It is applied to contaminated aquifers or sediments. High-pressure air is injected into the sediments, usually combined with the addition of nutrients, increasing the oxygen concentration, and enhancing biodegradation processes. Biosparging is a highly effective [116] and affordable technique, often cheaper among other remedial alternatives [117] due to the inexpensive and low-maintenance equipment that is used. It is not labor-intensive since it can be left unattended for long periods of time. The effectiveness of biosparging depends on the permeability of the sediments and the biodegradability of the contaminants [118]. High permeability sediments, such as sand and gravel, allow air to penetrate deeper and come in contact with remote microorganisms [119]. This capacity makes biosparging an attractive technique for marine sediment bioremediation, however an in situ application faces difficulties due to the dynamic profile of the environment. Biosparging cannot be applied in sites where high concentrations of inorganic salts, heavy metals, or organic compounds are present, as microbial growth is hindered [117].

###### 4.1.3. Coarse and Fine Bubble Diffusers

Diffusers are an ever-evolving technology for providing oxygen for bioremediation purposes in different environmental settings. Depending on the generated bubble size, diffusers can significantly enhance the aeration effectiveness and can come in a wide variety of geometries, sizes, and shapes (tube, disc, plate, dome) to meet specific requirements of a process. Their cost is relatively low compared to other aeration systems. The material of construction shapes the size of the bubbles produced. Membrane (ethylene propylene diene terpolymer, silicone, nitrile rubber, thermoplastic polyurethane, etc.) and ceramic air diffusers for example, can provide significantly smaller air bubbles, even in the scale of

nanometers. Each type of diffuser comes with different specifications regarding the service area ( $\text{m}^2$ ), the aeration volume ( $\text{L}/\text{min}$ ) and the oxygenation capability ( $\text{kg O}_2/\text{h}$ ), directly linked to the size of the bubbles that are formed and the air flowrate that the system can have during operation. These technical characteristics, however, refer to water or wastewater testing and little is known for the efficiency of air diffusers when they are used for sediment aeration. Out of the different air diffuser configurations, the most suitable to come in contact with sediments and provide oxygen are the tube diffusers, placed on top of the sediments. Diffused-air system aerators use a low-pressure, high-volume air blower to provide air to diffusers. The aeration efficiency of the diffusers is interchangeably linked with depth of application and flow rate of air [120]. As most of the equipment subjected to contact with microorganisms and redox compounds, the diffusers may exhibit erosion, biofouling, clogging of the pores, subsequently resulting in decreased efficiency and additional expenses for maintenance and cleaning. Diffusers can be categorized into two broad categories based on the type of bubbles they create: coarse bubble diffusers and fine bubble diffusers.

#### Coarse Bubble Diffusers

Coarse bubble diffusers produce bubbles of diameters greater than 10 mm [121]. Due to their size, coarse bubbles are not indicated for aeration purposes rather than mixing actions. Produced bubbles have a very limited time when introduced in water. They tend to coalesce and quickly escape from the water column, providing minimum residency time. For this purpose, coarse bubble diffusers are selected in applications where the medium presents high viscosity, such as the wastewater treatment. In general, coarse bubble diffusers' operation is cost and energy intensive. A more viable option of fine bubble diffusing systems has emerged and tends to eliminate the use of these diffusers. The only advantage of coarse bubble diffusers over the respective fine bubble ones is that due to larger pores, clogging could be avoided [119].

#### Fine Bubble Diffusers

Fine bubbles present a higher oxygen transfer efficiency (OTE); hence, they have gained a lot of attention against coarse bubble aeration systems. Their small size, typically less than 2 mm [122], provides a large surface area and enables higher residency time in the water due to less buoyancy forces [123]. Fine bubble diffusers are made of fine porous media such as ceramics, perforated membranes and porous plastics [124]. The advantages of smaller sized bubbles have led to the development of ultrafine pore diffusers which can produce bubbles in the scale of  $\mu\text{m}$  and  $\text{nm}$ . A more comprehensive overview of ultrafine (micro-nano) bubbles, which are nevertheless not produced by diffusers, is presented in Section 4.1.5.

#### 4.1.4. Injectors

Injectors are used to provide air or oxygen into the sediments and aquatic environments. For soil remediation, a typical air injection system consists of an air compressor to create the appropriate pressure drop for sparging, air filters to prevent the compressor from dust particles or produced vapors that include contaminants, control valves to regulate flowrate, and vertical injection wells, 2.5–10 cm in diameter and 30–60 cm deep, in which the appropriate piping is set for injection [125]. This setup constitutes a direct injection method in which the reagents are injected directly into the subsurface [126]. In aquatic environments, injectors are used for the recirculation of water, an injection method in which groundwater is extracted, mixed with air or oxygen and other nutrients and then reinjected from another injection well or from the same well (pull-push method). Direct injection, on the other hand, is only effective at sites with moderate groundwater flow, otherwise good distribution of the air within the plume is not achieved [127]. These methods mix the air with water, providing an oxygen rich jet which can promote efficient horizontal mixing of the aquifer but fails to promote the dissolution of oxygen vertically in the water column. This application is also constrained by the sediment characteristics: large constituents such as gravels or cobbles inhibit the application of this method [127].

#### 4.1.5. Micro-Nano Bubbles Technology

The use of micro and nano bubbles has extended beyond the diffusers. Their special characteristics have made them an attractive choice for use in air sparging as well, for bioremediation purposes [128]. Microbubbles diameter ranges between 10–50  $\mu\text{m}$  and for nanobubbles is less than 200 nm [129]. When both types of bubbles are introduced into a system, their diameter ranges between 0.1–50  $\mu\text{m}$  [130]. Research has shown that nanobubbles in water have a life expectancy that can reach up to weeks and even months in some cases [131]. Bubbles with diameters in the scale of microns offer a large surface area, thus being ideal for enhancing the oxygen transfer efficiency. Nanobubbles offer other advantages as well. They are found to be negatively charged [132], which prevents coalescence between the bubbles due to repulsion forces, resulting in high mass transfer efficiency and promoting self-pressurization dissolution [133]; their high inner pressure leads to fast diffusion of entrapped gases, which leads the bubbles to shrink even further and finally collapse, providing a large number of free radicals [128]. Specially designed generators have been developed over the years to produce tiny bubbles of air that can apply in a wide range of operations; from pharmaceuticals to wastewater treatment and can be categorized into two types. First the gas-water circulation type, where gas is introduced into a water vortex and breaks down into bubbles and second, the pressurized dissolution type, where about 5% of gas is under pressure dissolved in water and upon depressurization, the gas escapes forming nanobubbles [134]. Nanobubbles technology has shown to significantly improve water quality in an urban river, causing an 8-fold increase in the DO concentrations [135].

#### 4.1.6. Mechanical Agitation

Mechanical agitation could be as important as aeration, since it can increase the rate of oxygen transfer from the air bubbles to the water column and also increase the rate of nutrient supply to microorganisms. Wave and tidal forces in the marine environment sometimes offer naturally the proper mechanical agitation needed to replenish oxygen in the water column and sediments. However, these forces are absent in closed aquatic environments, such as lakes, ponds, and ports; hence, it is important to provide some kind of agitation for better oxygen distribution. In this case, pumps and paddle wheels are preferable for aeration but require a great amount of energy for throwing large quantities of water into the air. Aeration systems with a high air or oxygen flowrate could also cause agitation: typical examples are the coarse bubble diffusers and direct-push injection wells.

#### 4.1.7. Active Nautical Depth

First introduced in the port of Emden in Germany in 1990 [136], Active Nautical Depth (AND) is a sediment management technology which evolved as an alternative to dredging for minimizing siltation and maintaining navigable ports and harbors. AND derives from the concept of Passive Nautical Depth (PND), an alternative way to determine the depth of ports using density parameters, by the fact that fluid mud is created in situ by mixing and aerating the mud at the bottom of the water column which makes it navigable and therefore increases the nautical depth. The method uses a low-power submerged dredge pump that fluidizes the mud by breaking the inter-particle bonds and transfers it into a hopper dredger where it is aerated by exposure to the atmosphere and placed back to the bottom of the water body. The resuspension and aeration promote the aerobic growth of microorganisms and the production of extracellular polymeric substances (EPS) which allow particles to be kept in suspension for longer [137] and the fluid remains navigable for weeks. AND has limitations regarding the sediment particle size: it can be applied to muddy substrates with a sand content up to 10% [138]. In cases of higher sand content, the hopper dredger can perform sand extraction. Besides sediment management, AND has recently gained attention as a biostimulation method for the degradation of contaminants found in ports, such as tributyltin, a toxic xenobiotic found in aquatic environments [139]. This is due to the fact that AND changes the physicochemical properties of the sediment while maintaining aerobic

conditions. However, sediment resuspension caused by this method, perturbs the aquatic ecosystem, therefore side-effects should be carefully examined prior to application.

#### 4.1.8. Floating Bioreactor

This aeration technique was first presented by Thomas et al. [107] and involves a floating device developed to treat sediments with organic pollutants by airlifting and by resettling the aerated sediments at the same place. The main objective of the process is not to move dredged sediments to an onshore facility for treatment or disposal, but to recycle them at the same place where they have been accumulated. It is a similar method to airlift suspension reactors used for biodegradation processes. An airlift pump lifts the sediments above the water table. Compressed air is introduced right above the inlet of the riser pipe, creating a stream of air bubbles aiding the upwards movement of sediment particles and water. A knockout tank is integrated in the system where volatiles are stripped and treated with granulated activated coal. The lifted and aerated sediment suspension can be treated on-site through conventional separation and dewatering techniques but also reintroduced to the bottom of the water body. The acceleration with oxygen rich air not only stimulates microbiological degradation effects but also strengthens precipitation by flocculation. Since suspension can have a negative impact on the aquatic ecosystem by spreading the contaminants, floating barriers are used to control sediments during extraction and resuspension. Resettling process can be controlled with fixed cone shields and silt curtains to avoid contamination of the water column.

#### 4.1.9. Modular Slurry System (MSS)

A modular slurry system was initially introduced in 2014 [27] in an effort to provide sufficient oxygen for the biodegradation of oil polluted marine sediments avoiding the ex situ practices that could cause contamination of adjacent aquifers. The containment of marine sediments and the introduction of air through a submerged reactor resulted in 98% removal of the total extracted and resolved hydrocarbons and a decrease in toxicity of the contaminants in the area treated, thus indicating an efficient stimulation of the indigenous hydrocarbon degrading microorganisms [27]. A year later, Capello et al. [140] applied the MSS with simultaneous addition of nutrients to sustain aerobic bioremediation, and the results supported the previous findings, suggesting that in situ aeration of contaminated marine sediments after capping could be a feasible approach.

#### 4.1.10. Module for the Decontamination of Units of Sediment (MODUS)

Another novel technology for the aeration of sediments was introduced in 2020 [141]. The so-called module for the decontamination of units of sediment (MODUS) is a bench-scale system designed to aerate the bottom of the water column through a laminar flow directed tangentially to the seabed, without perturbing the top sediment layer. In a different approach, this technology could cause the oxygenation of sinking organic matter to the bottom of the sea or other aquatic ecosystems, resulting in the formation of a bio-oxy layer (bio-ox-capping) which is the development of oxygen-rich sediment layers. Left undisturbed, this layer would promote the aerobic metabolic activities of the indigenous microbiome and would restore the ecosystem prior to contamination toxicity.

### 5. Aerobic Bioremediation of Sediments—Case Studies

Engineered bioremediation was used extensively for the first time in Alaska shorelines after the Exxon Valdez oil spill in 1989, by means of the application of oleophilic and slow-release fertilizers [142–144], enhancing the biodegradation rates of oil. Since then, bioremediation has been applied to treat shallow polluted sediments in a number of situations. Early field demonstrations for sediment bioremediation in coastal environments and shorelines have been previously reviewed by Swannell et al. [145], and in salt marshes by Zhu et al. [146]. Table 1 presents the most recent field applications on aerobic sediment bioremediation from oil contaminants with focus on case studies that involve oxygen amendments and aeration.

**Table 1.** Recent field applications on aerobic sediment bioremediation from oil contaminants with focus on case studies that involve oxygen amendments and aeration.

Case Study	Location	Aquatic Environment	Type of Treatment <sup>1</sup>	Additives	Sediment Characteristics	Application Point	Duration of Application	Application Rate	Effectiveness	Reference/Year
1	Harbor of Marghera, Venice Lagoon, Italy	Port	FA+O	Oxygen	Quaternary sediments (unconsolidated sand, silt, clay, peats)	50 cm above the sediments	28 months	12 h/day continuous operation	Reduction of heavy metal content	Bonardi et al. [147], 2006
2	Arsenale shipyard, Venice Lagoon, Italy	Shipyard	FA+O	Oxygen	unconsolidated sand, silt, clay, peats	Sea floor	6 months	During the night	Reduction of heavy metal content. Recovery of the water body above the sediments	Bonardi et al. [148], 2007
3	Kerguelen Archipelago, The Grande Terre beach	Beach	BS	Slow-release fertilizer (Inipol EAP-22 or fish composts)	sand	-	3 years	-	Complete removal of aliphatic hydrocarbons after 6 months	Delille, Delille and Pelletier [149], 2002
4	Rybnik water basin, Poland	-	BS	Calcium peroxide	-	-	150 days	100 g/m <sup>2</sup> and 200 g/m <sup>2</sup>	Effective PAH removal	Kostecki and Mazierski [150], 2008
5	Shedu River, Jiangsu, China	River	BA+BS	Microbial activated beads	-	On top of the sediments	45 days	1.77–2.12 kg/m <sup>2</sup>	Good removal efficiency of pollutants	Fu et al. [151], 2018
6	Fish Farm, 2 nm offshore Porto Palo (Agrigento, Italy) in the Sicily channel (Med Sea)	Fish Farm	BA BS BA + BS	Biovase Oxygen Releasing Compounds	-	-	5 months	0.5 kg/m <sup>2</sup> Biovase 1 kg/m <sup>2</sup> ORC	Higher bacterial density and enzymatic activity	Vezzulli, Pruzzo and Fabiano [96], 2004
7	Bohai Sea, China	Sea	BA	Zeolite carrier with a polymer coating	-	Ocean floor	70 days	370 tons of remedy agents	Over 50% oil degradation	Zhao et al. [22], 2018
8	Bohai Sea, China	Sea	BA	Zeolite carrier with a polymer coating	-	Ocean floor	210 days	487 tons/km <sup>2</sup> of remedy agents	Increased oil biodegradation	Wang et al. [21], 2020
9	Inter-tidal foreshore of Pulau Semakau, Singapore	Beach	BS	Slow-release fertilizer (Osmocote) Slow-release fertilizer + oil sorbent biopolymer (Chitosan)	75.16% sand, 24.73% silt and 0.11% clay	Mixed on the top sediments	105 days 95 days	1.2% Os ( <i>w/w</i> ) and 0.1% chitosan (ChS)	Accelerated PAH degradation	Xu et al. [37], 2004; Xu et al. [38], 2005

Table 1. Cont.

Case Study	Location	Aquatic Environment	Type of Treatment <sup>1</sup>	Additives	Sediment Characteristics	Application Point	Duration of Application	Application Rate	Effectiveness	Reference/Year
10	St. Lawrence River at Ste. Croix, Quebec, Canada	Shore	BS Ph	Inorganic Fertilizers (ammonium nitrate NH <sub>4</sub> NO <sub>3</sub> and monobasic calcium phosphate Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> )	-	2–3 cm	455 days	2.85 kg of NH <sub>4</sub> NO <sub>3</sub> or 6.06 kg of NaNO <sub>3</sub> , and 1.22 kg of Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> ·H <sub>2</sub> O per plot	No difference to natural attenuation	Venosa et al. [152], 2002
11	Bullwell Bay, Milford Haven, UK	Beach	BS	Inorganic Fertilizer (Sodium Nitrate NaNO <sub>3</sub> and Potassium phosphate KH <sub>2</sub> PO <sub>4</sub> ) Slow-release fertilizer	shingle, pebble, clay	<10 mm	2 months	Inorganic Fertilizer (1.15 kg NaNO <sub>3</sub> and 0.08 kg KH <sub>2</sub> PO <sub>4</sub> in 9 L seawater/plot/week Pellet slow-release fertilizer single application	Increased heavy fuel oil degradation in both cases	Swannell et al. [153], 1999
12	Stert Flats, Somerset, United Kingdom	Mudflat	BS	Inorganic fertilizer (fertilizer grade Sodium Nitrate NaNO <sub>3</sub> and Potassium phosphate KH <sub>2</sub> PO <sub>4</sub> )	fine sand	15 cm	108 days	Weekly for a month, every 2 weeks thereafter	Significant enhancement of light crude oil bioremediation	Swannell et al. [154], 1999
13	Stert Flats, Somerset, United Kingdom	Mudflat	BS	Slow-release fertilizer Liquid inorganic fertilizer	fine sand	10 cm	~1 year	Weekly application of liquid fertilizer	Significant enhancement of light crude oil bioremediation	Röling et al. [155], 2004
14	Fisherman’s Landing Wharf, Gladstone Australia	Mangrove <i>Rhizophora stylosa</i> Salt Marsh	BS + FA BS + FA + CD	Aquarium airstones Osmocote Tropical Corexit 9527	-	2–3 cm	270 days	100 L/min of air for 4 months Osmocote Tropical added in the beginning and after 6 months	1 to 2-month lag time 1000-fold increase in alkane degraders	Ramsay et al. [42], 2000; Duke et al. [50], 2000; Burns, Codi and Duke [156], 2000
15	San Jacinto Wetland Research Facility (SJWRF), San Jacinto River near Houston, Texas	Wetland	BS	Inorganic Nutrients (diammonium phosphate (NH <sub>4</sub> ) <sub>2</sub> (HPO <sub>4</sub> )) Inorganic Nutrients + Alternative Electron Acceptor (Potassium Nitrate KNO <sub>3</sub> )	-	On top of the sediments	140 days	Inorganic Nutrients 40 mg N/kg dry sediment weight Electron Acceptor 100 mg N–NO <sub>3</sub> –/kg dry sediment weight bi-weekly broadcast spreading	Enhanced biodegradation rates	Mills et al. [157], 2004

Table 1. Cont.

Case Study	Location	Aquatic Environment	Type of Treatment <sup>1</sup>	Additives	Sediment Characteristics	Application Point	Duration of Application	Application Rate	Effectiveness	Reference/Year
16	San Jacinto Wetland Research Facility (SJWRF), San Jacinto River near Houston, Texas	Wetland	BA BS	Dry, wheat-bran-based powder containing a large consortium of hydrocarbon-degrading bacteria dry, wheat-bran-based (plus non-ionic surfactant) powder containing large numbers of oil-degrading microorganisms Inorganic fertilizer (diammonium phosphate (NH <sub>3</sub> ) <sub>2</sub> HPO <sub>4</sub> )	-	On top of the sediments	152 days	Applied 5 times (Days 4, 7, 11, 18, 28) Applied twice (Days 4 and 28) Broadcast spread prior to microorganisms	No additional response from exogenous microbes No significant differences to intrinsic bioremediation	Simon et al. [18], 2004
17	Pointe au Chien Wildlife Management Area in Terrebonne Parish, LA	<i>Spartina alterniflora</i> salt marsh	BS Ph	Ammonium Nitrate (NH <sub>4</sub> NO <sub>3</sub> ) Time-release Urea	-	On top of the sediments	180 days	Ammonium Nitrate 60 g N/m <sup>2</sup> Urea 30 g N/m <sup>2</sup>	Alkane degradation rates were not enhanced	Tate et al. [44], 2012
18	St. Lawrence River at Ste. Croix, Quebec, Canada Conrod's Beach, on the Eastern Shore of Nova Scotia, Canada	Freshwater wetland <i>Spartina alterniflora</i> salt marsh	Ph BS + T	Ammonium Nitrate (NH <sub>4</sub> NO <sub>3</sub> ) Sodium Nitrate (NaNO <sub>3</sub> ) and Orthophosphate nutrients (Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> ·H <sub>2</sub> O)	-	2–3 cm	65 weeks	1 kg-N and 0.3 kg-P per plot applied weekly 1.28 kg and 0.55 kg/plot applied when N concentration <5 mg/L	Inorganic nutrients can accelerate hydrocarbon degradation when oil is present mostly on the surface	Venosa et al. [158], 2002
19	Conrod's Beach, on the Eastern Shore of Nova Scotia, Canada	<i>Spartina alterniflora</i> salt marsh	BS Ph	Ammonium Nitrate (NH <sub>4</sub> NO <sub>3</sub> ) and Orthophosphate nutrients (Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> ·H <sub>2</sub> O)	-	On top of the sediments	20 weeks	0.45 kg-N and 0.135 kg-P per plot applied on days 0, 50 and 82	Alkane degradation enhancement, no effect on PAH degradation	Garcia-Blanco et al. [116], 2007

Table 1. Cont.

Case Study	Location	Aquatic Environment	Type of Treatment <sup>1</sup>	Additives	Sediment Characteristics	Application Point	Duration of Application	Application Rate	Effectiveness	Reference/Year
20	St. Lawrence River at Ste. Croix, Quebec, Canada	<i>Scirpus pungens</i> freshwater shoreline	Ph BS + T	Ammonium Nitrate (NH <sub>4</sub> NO <sub>3</sub> ) Sodium Nitrate (NaNO <sub>3</sub> ) and Orthophosphate nutrients (Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> ·H <sub>2</sub> O)	sandy loam (58% sand, 32% silt and 10% clay)	1–2 cm	21 weeks	1 kg-N and 0.3 kg-P per plot reapplied when N concentration <5 mg/L	No significant biodegradation enhancement	Garcia-Blanco et al. [159], 2001
21	Sveagruba, Spitsbergen, Svalbard, Norway	Arctic Shoreline	BS T BS + T	Soluble Fertilizer (ammonium nitrate NH <sub>4</sub> NO <sub>3</sub> and superphosphate Ca(H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> ) Slow-Release Fertilizer (Inipol SP1) Ferrous sulfate Yeast extract	Site 1: 41% pebble, 16% granules, 18% coarse sand, 25% sand/mud (Low energy); Site 2: 53% pebble, 11% granules, 13% coarse sand, 23% mud (moderate energy) Site 3: 75% pebble, 5% granules, 2% coarse sand, 18% sand/mud (locally high energy)	On top of the sediments 2–3 cm penetration	400 days	100 g/m <sup>2</sup> ammonium nitrate 10 g/m <sup>2</sup> superphosphate 1 g/m <sup>2</sup> ferrous sulfate 0.1 g/m <sup>2</sup> yeast extract (Day 0) 140 g/m <sup>2</sup> Inipol SP1 1 g/m <sup>2</sup> ferrous sulfate 0.1 g/m <sup>2</sup> yeast extract (Day 7) 100 g/m <sup>2</sup> Inipol SP1 (Day 23) 50 g/m <sup>2</sup> ammonium nitrate 5 g/m <sup>2</sup> superphosphate 1 g/m <sup>2</sup> ferrous sulfate 0.1 g/m <sup>2</sup> yeast extract 70 g/m <sup>2</sup> Inipol SP1 (Day 58)	Tilling did not clearly contribute to the removal of oil within the intertidal sediments Biostimulation increased the biodegradation rates in the intertidal sediments	Prince et al. [113], 2003; Owens et al. [114], 2003; Sergy et al. [115], 2003
22	N/A	Harbor	FA	Air	-	-	12 months	5–10 L/h	TPH removal up to 60–75% PAH removal up to 75–85%	Thomas et al. [107], 2008
23	Upper Main Harbor, Frankfurt/M. Germany	Harbor	BS	Hydrogen Peroxide and Fenton's reagents	Fine-grained (80% clay and silt)	Injected at the base of the sediment body	-	0.16 L/h of 1% peroxide solution (5 g/kg sediment) per screen	No degradation of organic pollutants was observed	Thomas et al. [107], 2008

Table 1. Cont.

Case Study	Location	Aquatic Environment	Type of Treatment <sup>1</sup>	Additives	Sediment Characteristics	Application Point	Duration of Application	Application Rate	Effectiveness	Reference/Year
24	N/A	Artificial fish ponds	FB	-	-	-	-	-	Under investigation	Thomas et al. [107], 2008; Thomas et al. [160], 2009
25	Virgen del Mar beach, at the north coast of Spain	Beach	BS	Oleophilic fertilizer S-200	Large and medium cobble stones overlying a mixed sand and gravel base	On top of the sediments	220 days	15.8 g N/m <sup>2</sup> and 1.37 g P/m <sup>2</sup> , according to C:N:P ratio of 120:10:1, applied on Day 0 and Day 20	The addition of fertilizer increased the biodegradation rate during the first 60 days	Jiménez et al. [41], 2006
26	Bahinas beach, coast of Asturias, Northern Spain	Beach	BS BA	Ammonium Nitrate (NH <sub>4</sub> NO <sub>3</sub> ) Ammonium Phosphate ((NH <sub>4</sub> ) <sub>2</sub> PO <sub>4</sub> ) Surfactant Commercial Bioaugmentation Products	Medium grain-size sand covered by pebbles and cobbles	On top of the sediments	45 days	C:N:P ratio 100:10:1 Weekly application	Bioaugmentation had positive effects on the degradation of the saturated fractions	Gallego et al. [23], 2008; Gallego et al. [161], 2007
27	Delaware Bay, United States	Shoreline	BS BA	Sodium Nitrate (NaNO <sub>3</sub> ) Sodium Tripolyphosphate (Na <sub>5</sub> P <sub>3</sub> O <sub>10</sub> )	-	On top of the sediments	-	2 kg of technical grade sodium nitrate (330 g of nitrogen) and 128 g of sodium tripolyphosphate applied everyday 30 L suspended mixed population of hydrocarbon degrading bacteria applied once a week	Biostimulation enhances the intrinsic rates of biodegradation Bioaugmentation has no significant effect	Venosa et al. [16], 1996
28	Kasumi-cho, Kinosaki-gun, Hyogo prefecture, Japan	Beach	BA	TerraZyme™	Rocks	On top of the sediments	8 weeks	250 kg	Significant enhancement on the biodegradation of heavy crude oil	Tsutsumi et al. [162], 2000

<sup>1</sup> Type of treatment: FA refers to forced aeration, O to oxygenation, BS to biostimulation, BA to bioaugmentation, FB to floating bioreactor, T to tilling, Ph to phytoremediation, and CD to chemical dispersants.

## 6. Conclusions and Perspectives

Site characterization is the primary concern prior to applying any bioremediation technique. Ex situ techniques that involve the dredging of contaminated sediments often come at higher costs, attributed to excavation and transport and present high probability of contaminant spreading during the removal. In situ techniques tend to be less invasive to the environment and cost-effective; nevertheless, their application must be in accordance with a number of factors. Sensitive ecosystems, for example, require a more delicate bioremediation approach.

The main consideration for treating sediments is to apply the most suitable and effective technique for oxygen supply. Oxygen being the limiting factor in sediment treatment often leads to long bioremediation times where the anaerobic metabolism of the indigenous microorganisms is employed. Different approaches have evolved to enhance anaerobic bioremediation; however, the time of the treatment still remains significantly slower than the one under aerobic conditions. Oxygen supply in some form seems to be the most efficient approach for the enhancement of the aerobic bioremediation. The quest for efficient aeration techniques has led in the development of novel technologies to provide alternatives for sediment bioremediation.

Among the sediments, marine sediments pose a real challenge for treatment due to the dynamic environment of the seas, linked with the high hydrostatic pressures of the deep ocean floor, and make any bioremediation implementation difficult. However, it is of great importance to address this matter because it poses great threat for marine ecosystems. Deep sea sediments bioremediation is still under investigation; nonetheless, suggestions can be made for sediment bioremediation in marine shorelines and commercial ports that directly affect human life.

Sediments in shorelines are known to be treated in situ with tilling during low tide, for better penetration of oxygen and nutrients, but other bioremediation techniques can be implemented. Phytoremediation could be the solution for sensitive environments such as salt marshes and mangroves. The use of aeration techniques is inhibited by tidal and wave activities of the shorelines. In the case of commercial ports, microporous aeration operated above the sediment or AND could be implemented for aerobic bioremediation purposes. The application of a MSS has proven the method's efficiency. Microporous aeration, coupled with specialized injection systems or diffusers, seems to be very promising in providing sufficient quantities of oxygen for enhanced aerobic bioremediation and, thus, could constitute a bioremediation strategy for the intricate environment of marine sediments.

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