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REVIEW

Microbe-Assisted Phytoremediation of Hydrocarbons in Estuarine Environments

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Abstract Estuaries are sinks for various anthropogenic contaminants, such as petroleum hydrocarbons, giving rise to significant environmental concern. The demand for organisms and processes capable of degrading pollutants in a clean, effective, and less expensive process is of great importance. Phytoremedition approaches involving plant/bacteria interactions have been explored as an alternative, and halophyte vegetation has potential for use in phytoremedition of hydrocarbon contamination. Studies with plant species potentially suitable for microbe-assisted phytoremediation are widely represented in scientific literature. However, the in-depth understanding of the biological processes associated with the reintroduction of indigenous bacteria and plants and their performance in the degradation of hydrocarbons is still the limiting step for the application of these bioremediation solutions in a field context. The intent of the present review is to summarize the sources and effects of hydrocarbon contamination in estuarine environments, the strategies currently available for bioremediation (potential and limitations), and the perspectives of the use of halophyte plants in microbe-assisted phytoremediation approaches.

Introduction

Salt marshes are complex coastal environments usually situated within estuarine systems. They represent dynamic habitats, developing along the coast line and inside estuaries and are characterized by high concentration of soluble salts (prevailing NaCl), relatively low diversity of species, and high biomass productivity [1, 2]. Estuarine salt marshes are among the most productive ecosystems on Earth [3] promoting plant and microbial activity [4–6], representing a preferential habitat for many organisms (fish, bird, and other wildlife) [7, 8] and providing important ecosystem services [9]. Salt marshes are highly dynamic areas, influenced by the joint action of water, sediment, and vegetation, providing a buffer zone between terrestrial and aquatic ecosystems in urban and industrial areas. They contribute to flood control and erosion prevention and may act as protective filters and final repositories for runoff pollutants, pathogens, and nutrients [1, 9, 10].

Salt marshes are sinks for various pollutants (e.g., metal and polycyclic aromatic hydrocarbon), receiving important anthropogenic inputs from urban areas, industries, and agricultural compounds, namely, polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene (HCB), hexachlorocyclohexane (HCH), and hexachlorodimethanonaphthalene (Dieldrin), as summarized in Table 1. Due to their ecological importance, the cleanup and recovery of these ecosystems is an issue of public concern.

Petroleum hydrocarbons (PHs) represent one of the most common groups of persistent organic pollutants in coastal and estuarine systems [22]. They are continuously released, persistent in the environment, toxic to many organism, and hazardous to human health [24]. This class of contaminants may be originated from industrial release products or from accidental spills [24]. Numerous studies indicate that salt marsh sediments are capable of retaining PHs and that the stimulation of microbial activity in the rhizosphere of plants can accelerate their biodegradation [25, 26]. Phytoremediation is one of the processes of hydrocarbon bioremediation, which has been intensively studied in the last decade. The continuous

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Table 1 Concentration of various pollutants detected in sediments at estuaries from around the world

Site	Pollutant^{a}	Concentration (μg/g)^{b}	Reference
Charleston Harbor Estuary, SC, USA	Al	8.54	[11]
	Cd	0.28	
	Cr	94.1	
	Cu	32.4	
	Fe	4.28	
	Pb	31.7	
	Mn	247.7	
	Hg	0.13	
	Ni	28.1	
	Zn	111.7	
Chesapeake Bay, MD, USA	Cd	17.6	[12]
	Cr	1,831.1	
	Cu	396	
	Fe	14.74	
	Mn	3,381.0	
	Ni	157.7	
	Pb	348.6	
	Zn	2,105.4	
	Hg	2,340.3	
	PAHs	23,322.8	
	PCBs	2,148.2	
San Francisco Bay, CA, USA	Cd	5.733	[13]
	Ni	39.677	
	Cu	126.076	
	Pb	265.229	
	Zn	295.527	
Humber Estuary, Eastern England	Cu	60	[14]
	Pb	127	
	Zn	344	
Mersey Estuary, UK	DDT	0.773	[15]
	PCBs	0.173	
	HCB	0.022	
	HCH	0.003	
	Dieldrin	0.167	
Suir Estuary, Ireland	Cu	23.194	[16]
	Pb	69.208	
Bay of Fundy, Canada	Hg	0.079	[17]
Mersey Estuary, UK	PAHs	3.766	[18]
	PCBs	1.409	
Salt marsh along coastal zone of Portugal	Hg	>10 to <0.5 ppm	[19]
Site	Pollutant^{a}	Concentration (μg/g)^{b}	Reference
Mitrena salt marsh, Sado, Portugal	PAHs	7.35	[20]
Yangtze River intertidal zone, China	Al	97213	[21]
	Fe	49627	
	Cd	0.750	
	Cr	173	
	Cu	49.7	
	Mn	1112	
	Ni	48	
	Pb	44.1	
	Zn	154	
Cávado River estuary, Portugal	PAHs	0.4023	[22]
Lima River estuary, Portugal	PAHs	800	[23]

[a] PAHs, polycyclic aromatic hydrocarbons; PCBs, polychlorobiphenyls; DDT, dichlorodiphenyltrichloroethane; HCB, hexachlorobenzene; HCH, hexachlorocyclohexane Dieldrin:hexachlorodimethanonaphthalene.

[b] Maximum concentration found in soil or sediments

release of hydrocarbons and their degradation products caused by anthropogenic activities around estuary areas leads to the necessity for efficient, inexpensive, and environmental friendly processes of hydrocarbon decontamination, such as phytoremedition. In that perspective, the interactions between halophytes, plants capable of growing in salt marshes, and their root-associated bacteria may play a relevant role in the remediation of contaminated areas. Cultivation-dependent and -independent approaches together with molecular approaches have been used to characterize plant–hydrocarbonclastic bacteria partnerships in the perspective of their exploitation for microbe-assisted phytoremediation. However, particular features of salt marsh ecosystems may impose difficulties in the process of implementation of these strategies in the field, and considerable research effort has been directed to a deeper understanding of halophyte-microbe interactions in oil-polluted environments.

The purpose of this paper is to summarize recent knowledge on the degradation of petroleum hydrocarbons in salt marsh sediments and to critically discuss the potential and limitations of microbe-assisted phytoremediation approaches for the recovery of oil-impacted ecosystems.

Hydrocarbons

Petroleum hydrocarbons (PHs) are common environmental contaminants and represent a serious problem in many parts of world [27–32], particularly in coastal and estuarine systems which may become seriously affected [33–35]. They are the principal components in a range of commercial products (e.g., gasoline, fuel oils, lubricating oils, solvents, mineral spirits, mineral oils, and crude oil). Petroleum products are a complex mixture of hundreds of hydrocarbon compounds, including various amounts of aliphatic and aromatic molecules. They enter and spread through the environment in many different ways [24]. Certain petroleum hydrocarbons are directly released in the water column, forming surface films while others tend to accumulate in the sediment.

PAHs are widespread in air, soil, sediment, surface water, groundwater, and runoff and are also found to accumulate in plants and aquatic organism [36–38]. In estuarine environments, polycyclic aromatic hydrocarbons (PAHs) are of great concern due to their potential for bioaccumulation, persistence, transport, toxicity, mutagenicity, and carcinogenicity [36, 39–41]. These compounds are introduced into estuarine environments from different sources and by a variety of processes (Fig. 1). Although PAHs are ubiquitous in the environment (fossil fuels, brush fires, volcanoes, and burning natural vegetation), anthropogenic activities, such as petroleum refining and transport activities dependent on the combustion of fossil fuels, are the major contributors to their release in the environment [39–42].

PAHs are classified as low molecular weight (LMW) and high molecular weight (HMW) according to the number and type of rings they have in the structure [43]. Based on their abundance and toxicity, 16 PAHs have been included in the list of priority pollutants of the US Soil Protection Agency [44]. Because of their high hydrophobicity and low lability, the process of PAHs remediation, especially in soils and sediments, is generally slow and expensive. The fate of PAHs in the environment depends on abiotic and biotic processes such as stabilization, landfarming (stimulation of indigenous microorganisms in the soil by providing nutrients, water, and oxygen), steam and thermal heating, chemical oxidation, bioremediation (bioaugmentation and biostimulation), and phytoremedition, which have been applied to the restoration of groundwater and soils/sediments [40, 45].

Phytoremediation of Hydrocarbons

Halophytes are defined as plants capable of completing their life cycle in salt concentrations around 0.200 M NaCl or even higher [46]. Moreover, many of these plants inhabit environments subject to constant flooding (e.g., coastal mangroves and salt marshes) [47]. Because estuaries and coastal habitats are highly exposed to environmental contamination, many studies addressed the use of halophytes in the phytoremediation of many pollutants (e.g., heavy metals, xenobiotics, and PHs) [48–52]. The physiological mechanisms that these plants use to tolerate salts are partly analogous to those involved in heavy metal resistance. Therefore, halophyte plants can accumulate metals, being therefore regarded as promising candidates for the removal or stabilization of heavy metals in polluted soils [53].

According to the fate of the contaminant or to the mechanism by which plants remediate contamination, these approaches are referred to as phytoextraction, rhyzofiltration, phytostabilization, phytovolatilization, phytodegradation, or rhizodegradation [54, 55]. Phytoextraction refers to the uptake, translocation, and accumulation of contaminants in the soil by plant roots into aboveground components of the plants. This technique involves the introduction of plants referred to as hyperaccumulators in polluted sites that after grown, are harvested. So, phytoextraction involves the repeated cropping of plants in contaminated soil until contaminant concentration

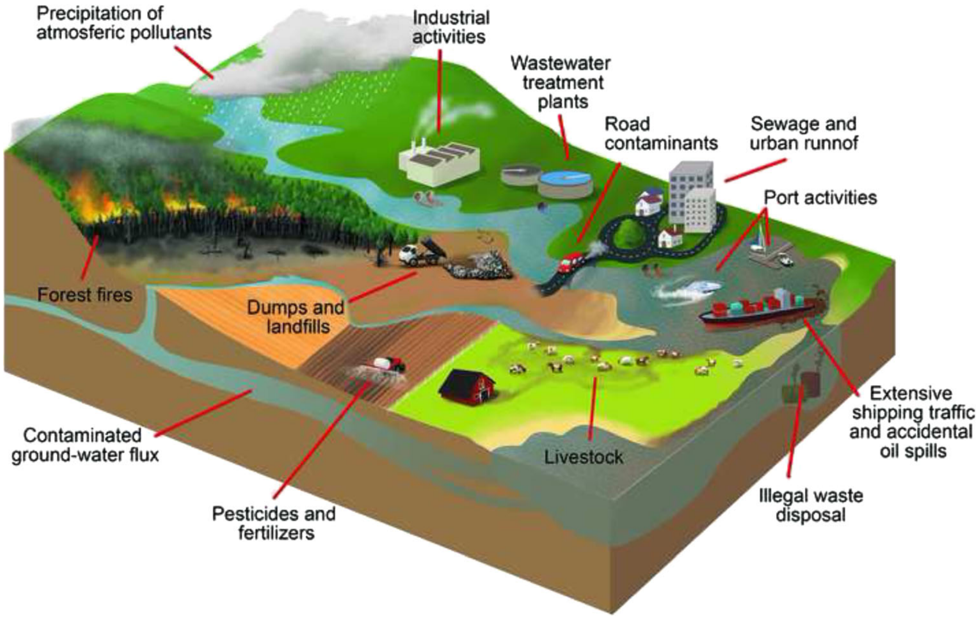

Fig. 1 Sources and processes involved in hydrocarbon release into estuarine ecosystems

decreases to acceptable levels. After harvesting, contaminated biomass needs treatments prior to disposal that can pass to secure landfills, incineration, or more recently thermochemical conversion processes (combustion, gasification, and pyrolysis) [56]. Rhyzofiltration involves the absorption or adsorption of contaminants through roots or other plant parts [54, 55]. In phytostabilization, plants reduce the bioavailability of contaminants immobilizing them in soil/sediment, reducing the mobility of contaminants and preventing migration to water or air [54, 55]. For the removal of low molecular weight compounds from soil, phytovolatilization is used. In this technique, plants volatilize contaminants that are biologically converting to gaseous species and releasing them through leaves via evapotranspiration process [54, 55]. Organic contaminants such as petroleum, PAHs, BTEX, TNT, chlorinated solvents, and pesticides are degraded only by plants (phytodegradation) or by microorganism and plants, in a process denominated as rhizodegradation [55].

Comparatively with application for the sequestration or removal of metals, studies involving halophytes for phytoremedition of PHs are still rather scarce. However, species of *Spartina*, *Salicornia*, *Juncus*, *Halonemum*, *Halimione*, and *Scirpus* have been tested for the remediation of hydrocarbons in wetlands with encouraging results (Table 2). The ability for PAH bioaccumulation was described for *Salicornia fragilis* shoots by a process of soil-to-plant transference that is dependent of exposure duration and pollution degree. High molecular weight PAHs were detected in aerial parts of the plant [58]. In a study conducted in greenhouse conditions, the use of *Juncus roemerianus* transplanted to salt marsh sediment contaminated with different diesel oil dosages was tested. The results revealed the reduction of PHs in *J. roemerianus* treatments, in relation to control sediments, suggesting that these plants may simultaneously contribute to the restoration and remediation of diesel-contaminated wetlands. Phytoremediation by *J. roemerianus* was even more effective for PAHs than for *n*-alkanes [48]. In an outdoor laboratory experiment (microcosm-scale), the potential of the salt marsh plants *Halimione portulacoides*, *Scirpus maritimus*, and *Juncus maritimus* for the remediation of soil contaminated with refinery waste was tested. Moreover, two situations are tested: (i) the use of each individual plant species or the use of an association of two plants (*S. maritimus* and *J. maritimus*) and (ii) soil with old contamination (crude oil) or a mixture of the old and recent (turbine oil) contamination. Combined transplants of *S. maritimus* and *H. portulacoides* plants were efficient in removing not only all the recent and old contamination, and the process was faster and more efficient than natural attenuation [49].

Despite existing evidence that the halophytes can be used successfully for the phytoremediation of estuarine areas, such as oil- or diesel-polluted sites (Table 2), there are still some limitations to the extensive use of this bioremediation approach. The efficiency of halophytes, as phytoremedition agents, depends on the plant species. For example, a comparative study of the efficiency of the salt marsh species *H. portulacoides*, *S. maritimus*, and *J. maritimus* for remediation of PHs revealed that plant species is determinant in the rate of hydrocarbon clearance and, more importantly, that different associations between these plants can interfere with or even inhibit the process [49].

Microbial Hydrocarbon Degradation

Contrasting with the prospective character of the use halophytes, the use of microorganisms for the clearance of hydrocarbons in the environment has long been regarded with interest. Through microbial activity, hydrocarbons are converted into carbon dioxide, water, and living biomass [60]. A diversity of bacteria, fungi, and algae has been characterized as to their capacity to degrade PAHs [36].

Microorganisms have been found to degrade PAHs via different catabolic pathways, such as anaerobic or aerobic metabolism, or co-metabolism which is important for the degradation of mixtures of PAHs and high molecular weight PAHs [38, 61, 62]. The initial step in aerobic metabolism of PAHs usually occurs via the incorporation of oxygen into aromatic rings followed by the systematic breakdown of the compound to PAHs metabolites and/or carbon dioxide. Anaerobic metabolism occurs via hydrogenation of aromatic rings [40, 62]. In co-metabolism, the range and extent of high

Table 2 Halophytes used for phytoremediation of hydrocarbons in estuarine areas

Plant	Result	Reference
Spartina alterniflora and *Spartina patens*	Restoration of oil-contaminated wetlands and accelerated oil degradation in soil	[57]
Salicornia fragilis	Intense bioaccumulation of PAHs from oil-polluted sediments in the shoots	[58]
Juncus roemerianus	Phytoremediation of diesel-contaminated wetlands	[48]
Halonemum strobilaceum	Phytoremediation of oil-polluted hypersaline environments via rhizosphere technology	[59]
Halimione portulacoides, *Scirpus maritimus*, and *Juncus maritimus*	Removal of petroleum hydrocarbons from soil	[49]
Scirpus triqueter	Enhanced biodegradation of diesel pollutants	[51]

molecular weight PAH degradation is influenced by an important interaction that transforms the non-growth substrate (PAHs) in the presence of growth substrates [62].

Physicochemical factors, such as soil type and structure, pH, temperature, electron acceptors, and nutrients, will affect microbial activity and determine the persistence of hydrocarbons (such PAHs) in polluted environments (Table 3). In estuarine areas, such as salt marshes, salinity fluctuations represent one of major challenges for hydrocarbon degradation that may even compromise the overall success of the process. There is an inverse relation between salinity and hydrocarbon solubility, with the consequent inhibitory effect of salinity on hydrocarbon bioremediation [71]. However, successful hydrocarbon degradation has been reported over a wide range of salinity values. A consortium of bacteria isolated from oil-contaminated sediments demonstrated the highest rate of hydrocarbon degradation with a salinity of 0.4 M NaCl, and the degradation was attenuated below and above this limit [72]. In a study with two bacterial consortia isolated from crude oil and mangrove sediments, the highest rate of degradation of aliphatic and aromatic hydrocarbons occurred in a salinity range between 0 and 0.171 M and decreased with increasing salinity [73]. Riis [74] reported diesel fuel degradation by microbial communities from saline soils in Patagonia up to a salinity of 2.997 M. Although hydrocarbon contamination is still persistent and recalcitrant in its nature, the fact that microbes from vegetated saline sediments can still actively degrade hydrocarbons in the presence of variable and relatively high concentrations of salt opens promising perspectives for microbe-assisted phytoremediation in estuarine areas.

Microbe-Assisted Phytoremediation

The microbial communities associated with plants and plant-microbe interactions established between them have a significant role in the physiology and health of the plant, exerted through inhibition of phytopatogens (e.g., antibiotic and siderophore production or nutrient competition), release of growth-promoting molecules, enhancement of nutrient availability, promotion of detoxification (e.g., sequestration, volatilization, and degradation of pollutants), and improvement of stress tolerance by induction of systematic acquired host resistance (Fig. 2).

The use of plants and their associated microorganisms for the removal of contaminants from the environment is based on the increase of microbial population numbers in the rhizosphere and/or endosphere and on the stimulation of their metabolic activity [75]. So, microbe-assisted phytoremedition represents a powerful emerging approach to sequester, degrade, transform, assimilate, metabolize, or detoxify contaminants from soil, sediment, or groundwater [54, 76].

Numerous bacteria found in association with plants are capable of degrading hydrocarbons, namely, PAHs, suggesting that indigenous rhizobacteria and endophytic bacteria may have potential for bioremediation of polluted sites [77]. In the particular case of estuarine environments, a diversity of hydrocarbon-degrading microbial populations has been found in association with the rhizosphere of salt marsh plants, where they actively contribute to hydrocarbon removal and degradation [23, 26]. The use of rhizosphere and phyllosphere (aerial portion of plants) of the halophyte *Halonemum strobilaceum* was also proposed for phytoremediation of oil-polluted hypersaline environments, via rhizosphere technology [59].

Rhizodegradation appears to be a particularly interesting phytoremediation process for the removal and/or degradation of organic contaminants, such as PH. The rhizosphere is defined as a zone directly influenced by plant root system. Plants provide root exudates rich in carbon sources, nutrients, enzymes, and sometimes oxygen, creating a favorable environment in which microbial activity is stimulated [75, 78, 79]. However, microbial interactions with plants are not limited to

Table 3 Factors affecting the biodegradation of petroleum hydrocarbons

Factor	Effect	Reference
Bioavailability	Composition and concentration of hydrocarbons affect the rate and extent of biodegradation	[40, 63, 64]
Temperature	Affects the physicochemical behavior of hydrocarbons (viscosity, diffusion, solubility)	[40, 63–65]
	Affects the physiology and diversity of microorganism	
pH	Inhibits microbial activity by regulating microbial metabolism	[40, 63, 66]
Nutrients	Availability of limiting nutrients (N, P, K, Fe) affects microbial growth and consequently biodegradation rates	[40, 63, 64, 67]
Oxygen	Despite the occurrence of biodegradation of hydrocarbons in anaerobic and aerobic conditions, oxygen depletion decreases biodegradation rates	[36, 40, 63]
Salinity	Changes in salinity promote alteration of the microbial population that affects biodegradation rates. High concentration of salt inhibits hydrocarbon degradation	[63, 68]
Organic matter	Promotes sequestration of contaminants, interfering in their availability	[36, 70]
Soil type and structure	Soil type influences the bacterial colonization and microbial activities and subsequently the efficiency of contaminant degradation	[69, 112]

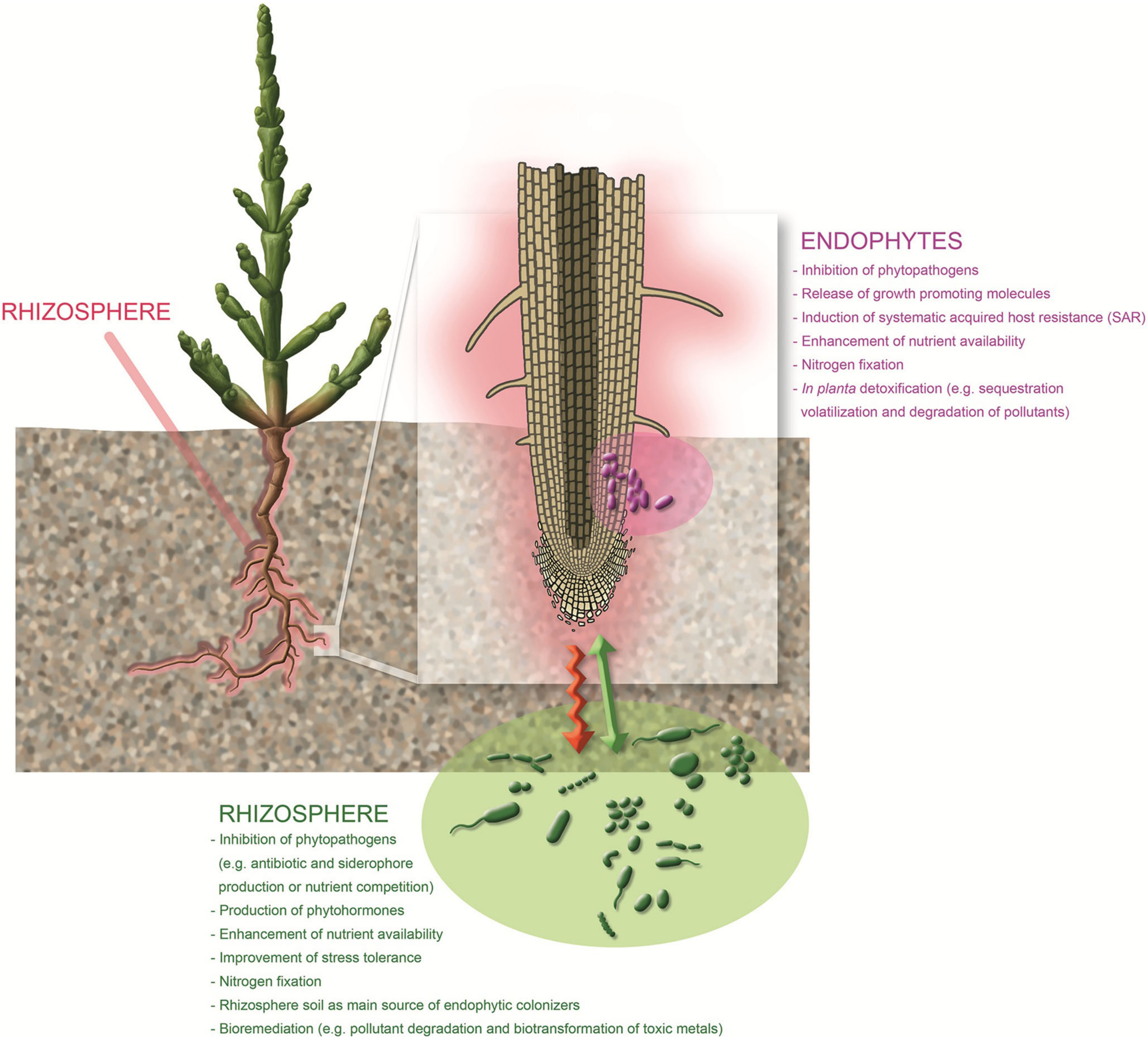

Fig. 2 Plant-microbe interactions and plant-growth-promoting effects of rhizosphere and endosphere bacteria

the rhizosphere; rather, they extend to the interior of the plant [80]. Endophytic-assisted phytoremedition, involving microorganisms that are capable of living within various plant tissues (roots, stems, and leaves), has been reported in recent years as successful in the degradation of some pollutants, such as explosives, herbicides, and hydrocarbons [77, 81, 82]. In fact, remediation of hydrocarbons by combined use of plants and rhizobacteria and endophytic bacteria have been widely described (Table 4). Genetically engineered endophyte microorganisms enhance the overall health of theirs hosts [92] and may indirectly improve biodegradation of contaminants in the rhizosphere. Experiments in which pea plants were inoculated with the naphthalene degrader *Pseudomonas putida* VM1441 (pNAH7) and exposed to naphthalene contamination revealed that naphthalene degradation rate (~40 %), seed germination, and plant transpiration were enhanced in inoculated plants than in non-inoculated controls. Moreover, inoculation resulted in an overall protection of the host plants from the phytotoxic effects of naphthalene [85]. A study carried out in a mangrove showed that nursery conditions and early microbial colonization patterns had long-term effect on the rhizosphere of transplanted mangroves. This phenomenon may have potential application for introducing new rhizocompetent bacteria carrying genes or plasmids to improve plant growth or bioremediation purpose (rhizoengineering) [93].

Several studies reported the enhancement of PH degradation in association with the presence of bacteria carrying PH degradation genes (Table 4). A high diversity of hydrocarbon

Table 4 Examples of successful approaches of remediation of hydrocarbons by combined use of plants and rhizo and/or endophytic bacteria

Rhizo- or endophytic bacteria	Plant	Gene (s)[a]	Plant growth promotion features[b]	Type of contaminant	Percen. Degradation[c]	Reference
Pseudomonas sp. GF3	*Triticum aestivum*	Unknown phenanthrene degradation gene		Phenanthrene	84.8 % in 80 days	[83]
Culturable n-hexadecane degraders	*Festuca rubra*	*alkB*, *ndoB*, *nidA*, *phnA*c and C2,3O		Mixture of hydrocarbons[d]	50 % in 4.5 months	[84]
Pseudomonas putida VM1441 (pNAH7)	*Lolium multiflorum*	*nah* (plasmid NAH7)		Naphthalene	40 % in 14 days	[85]
Pseudomonas strains, UW3 and UW4	*Lolium perenne*, *Festuca arundinacea*, and *Secale cereale*		ACC deaminase, siderophores and IAA producing stain	Oil refinery	65 % in 3 years	[86]
Azospirillum brasilense SR80	*Secale cereale* and *Medicago sativa*		IAA producing stain	Oil sludge	approx. 70 % in 120 days	[87]
Pantoea sp. strains, ITSI10 and BTRH79; *Pseudomonas* sp. strains, ITRI15 and ITRH76	*Lolium multiflorum* and *Lolium corniculatu*s	Unknown alkane degradation gene; cytochrome P450 alkane hydroxylase and *alkB* gene		Diesel fuel	>57 % in 90 days	[88]
Gordonia sp. S2RP-17	*Zea mays*		ACC deaminase and siderophores	Diesel fuel	96 % in 46 days	[89]
Pantoea sp. strains, ITSI10 and BTRH79; *Pseudomonas* sp. MixRI75	*Lolium multiflorum*	Unknown alkane degradation gene; cytochrome P450 alkane hydroxylase and *alkB* gene	ACC deaminase	Diesel fuel	approx. 79 % in 93 days	[90]
Pseudomonas sp. SB	*Testuca arundinacea*		ACC deaminase, siderophores and IAA producing stain	Oil	85 % in 120 days	[91]

[a] *alkB*: alkane monooxygenase; *ndoB*: naphthalene dioxygenase; *nidA*: naphthalene inducible dioxygenase; *phnAc:* phenanthrene dioxygenase; C2,3O: catechol 2,3 dioxygenase; nah: encoded same proteins for both upper and lower pathway of naphthalene degradation

[b] ACC: 1-aminocyclopropane-1-carboxylate deaminase activity; IAA: indole-3-acetic acid

[c] Maximum degradation obtain from sediments or soils in each study

[d] Hydrocarbon contaminated site located in south-eastern Saskatchewan, Canada (approx. 3000 to 3500 ug/g)

degradative genes, such as alkane monooxygenase (*alkB*), naphthalene dioxygenase (*ndoB*), phenanthrene dioxygenase (*phnAc*), and cytochrome P450 alkane hydroxylase, has been detected in plant microhabitats (rhizosphere and endosphere) [82, 84, 85]. In fact, the monitoring of gene abundance and expression during phytoremedition of contaminated sites can give indications about the persistence and functional activity of inoculated microorganisms [94]. A study conducted at a long-term phytoremediation field site revealed that both rhizosphere and endophytic communities showed substantial interspecies variation in hydrocarbon degradation potential and activity levels, with an increase in catabolic genotypes in specific plant treatments [82].

Recently, it was suggested that for certain phytoremedition approaches, it may be essential or at least important that bacteria also act as plant growth promoters, in addition to their pollutant-degrading activity. In experiments with Italian ryegrass, plant biomass production and alkane degradation were significantly enhanced by inoculation with bacterial strains expressing hydrocarbon-degrading genes (e.g., *alkB*) as well as plant-promoting activity (1-aminocyclopropane-1-carboxylate (ACC) deaminase activity) [90, 95]. So, the combined use of plant and bacteria can be exploited to relieve plant stress, and enhance bioremediation of PH-contaminated sites.

Current Limitations to the Microbe-Assisted Phytoremediation of Hydrocarbons

Microbe-assisted phytoremediation has been broadly tested for the degradation or sequestration of hydrocarbons in estuarine environments. Despite being considered an inexpensive, sustainable, and environment-friendly technique, phytoremedition is not exempt of controversy, and the success of this type of approach is significantly affected by environmental factors and particular features of each ecosystem. One major limitation is time, considering that successful phytoremediation is a process that goes on for long periods which is partially determined by the slow growth and phenological (or life) cycle of plants, the limited depth of root system, and the fact that many plant species are sensitive to the contaminants that are being remediated [75, 96].

The bioavailability of petroleum hydrocarbons is another important factor in the success of bioremediation, and it can be significantly affected by soil type and organic matter content. Water content (affects the availability of oxygen required for aerobic respiration), temperature, and nutrient availability (influences the rate and extent of biodegradation) are relevant determinants of the efficiency of the PH bioremediation process [78]. The competition for nutrients between plants and microorganisms can be a restriction to the remediation efficiency. A reduction in microbial abundance and an attenuation of degradation of higher molecular weight PAHs in sediments were observed in *H. portulacoides* banks, and this effect was associated to nutrient limitation [22]. Therefore, fertilization may be required for optimal rhizoremediation of hydrocarbons.

Although a wide range of hydrocarbon-degrading bacteria have been isolated from contaminated environments, little is known about the stability of the association with salt marsh plants and the success of the re-introduction of plant-bacteria systems for potential phytoremediation processes in saline sediments. The fact that these biotopes are colonized in a particular type of plants, well adapted to flooding and to salinity fluctuations, reinforces the need to incorporate basic knowledge on their interaction with sediment microbes in the design of phytoremediation approaches. The ability to monitor the survival and efficiency of hydrocarbon degradation of inoculated strains is essential for the in-depth understanding of the network of relations established between sediments, plants, and microbes that underlies microbe-assisted phytoremediation.

Monitoring Plant-Bacteria Interactions Involved in Microbe-Assisted Phytoremediation

The efficient colonization of plants by microbial pollutant degraders is an essential contribution for plant survival and hydrocarbon degradation [90, 97]. Despite the lack of knowledge on inoculation and bacterial colonization of halophyte plants, the monitoring of plant-bacteria interaction in hydrocarbon-polluted sites has been addressed by different approaches. In bioremediation, the use of culture-dependent methodologies, such as dilution plating on agar plates containing antibiotics, the most-probable-number (MPN) method, and direct counting, are insufficient for an accurate and sensitive monitoring of the inoculation and colonization processes [98]. Molecular techniques, including polymerase chain reaction (PCR), real-time PCR, and DNA hybridization, reporter genes, or genetically marker microorganisms (biomarkers) have been used to check on microbe survival, efficiency of colonization, and activity [77, 90, 94, 99, 100]. For example, antibiotic resistance and green fluorescent protein (*gfp*) genes have been proposed as useful tools for monitoring the colonization of bacterial endophytes, inoculated in poplar trees [100]. Endophyte colonization has also been monitored with the use of *gus*A marker gene encoding the enzyme β-glucuronidase. A *gus*-marked strain, *Burkholderia phytofirmans* PsJN, was inoculated in seeds of ryegrass (*Lolium multiflorum* Lam.). *B. phytofirmans* PsJN:gusA10 revealed that this bacterium has the ability to colonize the rhizosphere and endosphere of ryegrass vegetation in a diesel-contaminated soil and generally improved plant biomass production and hydrocarbon degradation [101]. Other study, using restriction fragment length polymorphism (RFLP), showed that *Enterobacter ludwigii* strains were able to efficiently colonize the rhizosphere and endosphere of Italian ryegrass, birdsfoot trefoil, and alfalfa. Moreover, *E. ludwigii* strains contain a cytochrome-P450-type alkane hydroxylase (CYP153), and the quantification and expression of these genes by real-time PCR indicate an active role in hydrocarbon degradation, in the rhizosphere and endosphere of all three plant species [95]. Quantitative PCR has emerged as a useful and rapid tool for monitoring catabolic genes during bioremediation processes. As an example, this technique was used for the assessment of hydrocarbon degradation activity of *Nocardia* sp. *H17-1* during remediation of crude-contaminated soil [102]. A similar approach was used to demonstrate that hydrocarbon degradation was associated with functional changes in microbial communities, in which high copy numbers of catechol 2,3-dioxygenase and naphthalene dioxygenase correlated with PAH mineralization [103].

Metagenomic pyrosequencing, which allows the recovery of a very large number of microbial sequences directly from environmental samples, has more recently emerged as a powerful technique to follow plant-microbe interactions during the bioremediation process [104, 105]. The sequences obtained can be compared with reference libraries, and then taxa present in an environmental sample can be identified with high confidence. The massive data sets generated provide information that can be used for a variety of applications, such as the comprehensive understanding of within-site and between-site variability of microbial communities and the impact of this variability in ecosystem-scale processes in salt marshes [106]. The pyrosequencing analysis of bacterial 16S ribosomal RNA (16S rRNA) gene fragments of different *Phragmites australis* rhizospheres revealed a trend in the variation of bacterial community structure during wetland degradation and identified sulfur and sulfate-reducing bacteria, nitrifying and nitrogen-fixing bacteria, and methane-oxidizing bacteria as crucial in the protection and ecological restoration of wetlands [107]. Recent bioremediation studies have used pyrosequencing analysis of bacterial 16S rRNA gene to describe microbial community dynamics in hydrocarbon-contaminated sites, thus providing basis for the development of strategies for monitoring remediation processes [108, 109]. For example, the relative abundance of Chloroflexi, Firmicutes, and Euryarchaeota was directly correlated with the presence of diesel [109].

Future Perspectives

Despite numerous limitations, phytoremediation and, particularly, microbe-assisted phytoremediation have undeniable advantages, and research must now specifically address the aspects that can allow the scaling up from laboratory to the field for the practical implementation of this approach.

Each salt marsh displays particularly biological, chemical, and physical characteristics that will ultimately determine the success of phytoremediation. Therefore, field studies, combined with laboratory approaches, are required for the understanding of the interplay of biological and chemical processes involved in microbe-assisted phytoremediation of oil-impacted sites.

Considering that plant-microbe interactions play a key role in the process of environment and *in planta* detoxification, (a) the identification of autochthonous hydrocarbon-degrading bacterial populations associated to salt marsh plants (rhizosphere and aboveground plant tissues), (b) the identification of degradative plasmids, and (c) the selection of petroleum-resistant plants are key issues for the success of environmental restoration. The detection of genes related to hydrocarbon degradation pathways in halophyte plants can be useful to screen for lineages of plants that can be used in efficient phytoremediation protocols. Moreover, these genes can be used for the genetic engineered design of plants for novel phytoremediation approaches for hydrocarbon-polluted wetlands and soils [110]. Recent plant biotechnology approaches involving the introduction of specialized bacterial endophytes in plants or the design of genetically engineered plants containing interesting bacterial genes [92, 111] create new perspectives for future phytoremedition protocols. Endophytic hydrocarbon-degrading bacteria may have a growth-promoting effect on the wild salt marsh halophyte plants and may be regarded as promising when field microbe-assisted phytoremediation approaches are envisaged.

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