Bioremediation of Contaminated Environments Using *Rhodococcus*

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Contents

Abstract Environmental pollution with anthropogenic organic compounds is the global problem of our planet. Bioremediation has a great potential to effectively restore polluted environments by using biodegradative activities of microorganisms. The genus *Rhodococcus* is a promising group of bacteria suitable for biodegradation of recalcitrant contaminants, such as petroleum hydrocarbons, chlorinated, nitrogenated, and other complex organics. *Rhodococcus* species are ubiquitous in pristine and contaminated environments, survive under harsh environmental conditions, compete successfully in complex bacterial populations, and therefore could be efficiently used in bioremediation applications. Some success in bioremediation of contaminated soils, waters, and air has been achieved using rhodococci either as bioaugmentation agents or members of indigenous microbial communities stimulated by nutrient and oxygen amendments. Laboratory and field-scale studies on *Rhodococcus* application in

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cleanup technologies are reviewed relating to in situ subsurface and groundwater remediation, on-site treatments of contaminated soils, sludges, wastewaters, and gaseous emissions.

1 Introduction

Environmental pollution with anthropogenic organic compounds has become one of the most urgent problems worldwide. This negatively impacts economical and social developments and poses significant threats to human health and natural biodiversity. Potential sources of organic contaminants include industrial leaks and spills, improper application of pesticides and fire retardants, negligent disposal of industrial and domestic wastes, and landfills and garbage dumps. Oil spillage and oily waste deposits represent the major part of hydrocarbon contamination in our planet. Petroleum hydrocarbons are widespread soil and groundwater pollutants resulting from leaking of underground storage sites, spillage from the storage tanks, and damaged pipelines. There are thousands of sites that have been seriously contaminated by petroleum products in oil-producing regions around the world (Etkin 2001; Ivshina et al. 2015a). Moreover, marine oil spills from crashed tankers are responsible for the massive contamination of seawater and shorelines. Apart from oil industry, major sources of hydrocarbon contaminants, such as alkanes and polycyclic aromatic hydrocarbons (PAHs), are coal-mining sites and coking plants, gas processing plants, solid fuels for domestic heating, aircraft and car exhausts, and forest fires. Also, chlorinated hydrocarbons such as chlorobenzenes, chlorophenols, and polychlorinated biphenyls (PCBs) that are used commercially for a variety of purposes, including production of solvents, paint additives, pesticides, fire retardants, and insulating fluids, represent a large proportion in long-term persistent contamination of soils and groundwater. It should be noted that soil contamination by organic compounds is a complex process and difficult to treat due to many reasons, for example, a tendency to sorption of contaminants into the soil matrix, low water solubility, and limited rate of mass transfer. Remediation activities are often hampered by remoteness and low accessibility of contaminated sites, harsh environmental conditions, as well as high pollution levels and large amounts of contaminated material to be treated. Bioremediation is considered to be a nondestructive, cost- and treatment-effective, and sometimes logistically favorable cleanup technology capable of accelerating naturally occurring biodegradation of contaminants through the optimization of limiting conditions (Alexander 1999). Currently, methods of biological remediation of contaminated sites gain ever increasing popularity due to their sustainability, relatively low cost, and environmental safety (Fig. 1).

Bioremediation of polluted environments is based on contaminant biodegradation, that is, metabolic abilities of microorganisms to transform or mineralize organic

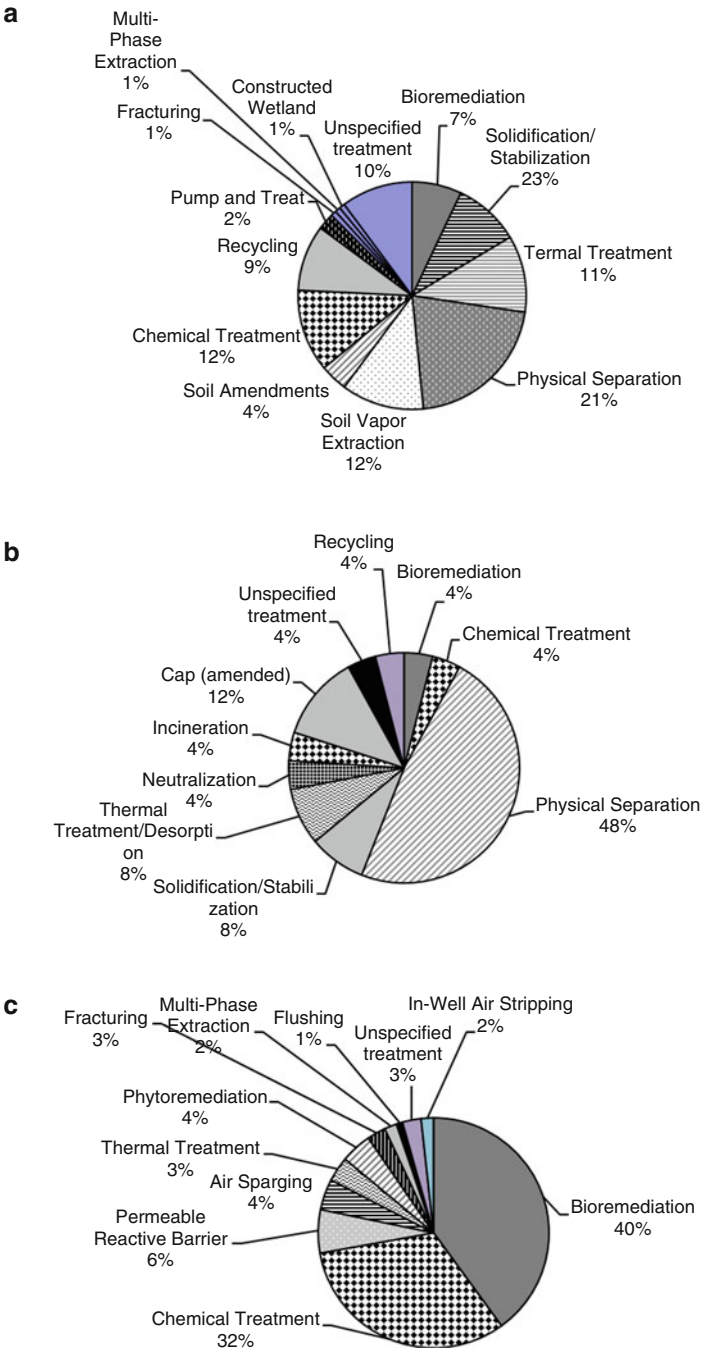

Fig. 1 Proportion of bioremediation among remediation technologies reported in the US EPA Superfund Remedy Report (15th Edition) for 2012–2014. (**a**) Source [soil, sludge, sediment, solid waste, debris, drummed waste, leachate, and any nonaqueous phase liquid both light (LNAPL) and

contaminants into less harmful, nonhazardous substances, which are further integrated into natural biogeochemical cycles (Alexander 1999; Whyte et al. 2001). In most cases, treatment of contaminated environments has involved biostimulation, addition of nutrients and other factors to stimulate spontaneous enrichment of the indigenous contaminant-degrading microbial population. However, there has been considerable debate over the efficacy of bioaugmentation (Aislabie et al. 2006), the addition of either indigenous or exogenous microorganism's cultures to enhance the remediation process. An application area for bioaugmentation could be contaminated environments deficient in microorganisms due to extreme climate conditions (e.g., polar and desert soils, low-oxygen groundwaters) or high levels of toxic contaminants (e.g., industrial waste dumps and wastewater streams). For example, low numbers of hydrocarbon-oxidizing bacteria in cold climate pristine soils coupled with short summer seasons may limit the spontaneous enrichment of oil-contaminated soils with autochthonous hydrocarbon oxidizers when biostimulation alone is applied (Ruberto et al. 2005). Bioaugmentation, in the context of bioremediation, should be considered as inoculation of contaminated soil or water with specific strains or consortia of microorganisms to improve the biodegradation capacity of the system for specific organic pollutant(s) (Alexander 1999). There are two fundamental approaches to bioaugmentation of a contaminated site. The first involves increasing the genetic diversity of the residential microbial community by inoculation with allochthonous microorganisms, which may enhance the catabolic potential, and thereby the biodegradation rate (Dejonghe et al. 2001). The second approach applied in most bioremediation projects involves a serial enrichment of indigenous microbial populations isolated from the contaminated samples using laboratory cultivation with the contaminant(s) as the sole carbon source. This enriched inoculum containing mostly fast-growing organisms with exceptional degradative capacities is then returned back to the site to increase the rate of biodegradation. Various factors are known to influence the success or failure of bioaugmentation; the predictability, however, is beyond our scope yet. In many cases, contaminated environments are hostile to the introduction of allochthonous microorganisms, and large numbers will never survive unless they have a selective advantage. The controlling environmental factors are both biotic and abiotic (for reviews, see van Veen et al. 1997; Watanabe and Hamamura 2003). So, assuming bioaugmentation as a feasible bioremediation approach, it seems to be important to find the "proper" microorganisms for the bioaugmentation consortium. These microorganisms should possess high biodegrading abilities, be highly competitive in natural bacteriocenoses, as well as be nonpathogenic and environmentally friendly.

The genus *Rhodococcus* was regarded as one of the most promising groups of microorganisms suitable for the biodegradation of compounds not readily transformed by other organisms. The biochemical potential of rhodococci has been increasingly explored because of their broad catabolic versatility and unique enzymatic capabilities

Fig. 1 (continued) dense (DNAPL)] treatment. (**b**) Sediment treatment. (**c**) In situ groundwater treatment. Figures generated from US EPA (2017)

(Van der Geize and Dijkhuizen 2004; Martínková et al. 2009; Ivshina et al. 2017). Xenobiotic compounds metabolized by rhodococci cover a wide range of structural groups, including aliphatic and aromatic hydrocarbons, oxygenated and halogenated compounds, nitroaromatics, heterocyclic compounds, nitriles, various pharmaceuticals, and pesticides. Many of these substrates are complex synthetic molecules with high chemical stabilities and toxicity. *Rhodococcus* species are ubiquitous in pristine and contaminated environments, possess remarkable metabolic activities, can persist under harsh environmental conditions, compete successfully in complex bacterial populations, and therefore could be considered as having great potential in bioremediation applications. The significance of rhodococci in environmental biotechnology was discussed in early reviews characterizing the genus *Rhodococcus* (Warhurst and Fewson 1994; Bell et al. 1998). This chapter describes the roles of rhodococci as members of natural hydrocarbon-oxidizing consortia and promising bioremediation agents and also reviews laboratory and field-scale studies on *Rhodococcus* application in cleanup technologies for contaminated environments.

2 Why Are Rhodococci Considered as Most Suitable for Environment Bioremediation?

Rhodococcus species are naturally present in diverse temperate and extreme environments. They can persist and grow in highly contaminated soils and waters and even under oxygen- and nutrient-limited conditions. *Rhodococcus* strains can be readily isolated from environmental samples and enriched in mixed or pure cultures by incubation with a particular contaminant of interest, which is important for preparing inocula for bioremediation. Their natural occurrence in contaminated environments, broad catabolic versatility, and physiological and ecological adaptations to extreme environmental conditions imply that they may play a significant role in both natural degradation of persistent pollutants and bioremediation of contaminated ecosystems.

2.1 Pristine and Contaminated Environments Are Common Habitats for Rhodococcus *Species*

Rhodococci have been isolated from a large variety of natural sources, including clean and contaminated soils and rocks, surface and groundwaters, and marine sediments, from animals and plants. *Rhodococcus* strains are often isolated from environments where hydrocarbons are present. Since petroleum hydrocarbons are most widespread contaminants of terrestrial and marine ecosystems, a large variety of studies were undertaken attempting isolation, characterization, and cleanup application of petroleum-degrading bacteria (for a review, see Van Hamme et al. 2003).

Some *Rhodococcus* species, particularly *R. rhodochrous* and *R. ruber*, are able to grow using gaseous hydrocarbons (such as propane, butane, and acetylene) as a sole carbon source (Ivshina et al. 1994). Ivshina et al. (1981) found large numbers of propane- and butane-degrading *R. rhodochrous* and *R. ruber* isolates in soil, rock, and groundwater associated with oil-bearing sites, suggesting that these gas-oxidizing rhodococci are indicative of the presence of subterranean hydrocarbon deposits and thus could be used in oil prospecting. Crude oil-contaminated soil and marine samples collected from different localities in Kuwait were screened for microorganisms capable of oil degradation (Sorkhoh et al. 1990). It was found that, among many bacterial isolates, *Rhodococcus* strains were the most abundant mesophilic hydrocarbon-oxidizing bacteria and most efficient oil degraders. Cold-tolerant *Rhodococcus* strains were isolated from oil-contaminated soils in Antarctica; they grew on a range of alkanes from hexane (C_6) through at least eicosane (C_{20}) and the isoprenoid compound pristane (2,6,10,14-tetramethylpentadecane) and retained metabolic activities at sub-zero temperatures of −2 °C (Bej et al. 2000). Several cold-tolerant *Rhodococcus* strains isolated from a deep Greenland glacier ice core grew rapidly at 2 °C (Miteva et al. 2004). A psychrotrophic *Rhodococcus* sp. from Arctic soil (Whyte et al. 1998) utilized a broad range of aliphatics (C_{10}–C_{21} alkanes, branched alkanes, and a substituted cyclohexane) present in diesel oil at 5 °C. The strain mineralized short-chain alkanes (C_{10} and C_{16}) to a significantly greater extent (by a factor of about 2–3) than long-chain alkanes (C_{28} and C_{32}) at 0 and 5 °C. The psychrotrophic halotolerant oil-degrading strain *Rhodococcus* sp. YHLT-2 isolated from gasoline-contaminated groundwater was able to grow at 7% NaCl and utilized short-chain alkenes (C_9–C_{12}) as well as a broad range of long-chain alkenes (C_{19}–C_{32}) present in crude oil at 4 °C (Ryu et al. 2006). The study of Mergaert et al. (2001) showed that a large proportion (34%) of facultative oligotrophic and psychrotrophic strains isolated from Arctic and Antarctic seawaters were grouped according to their fatty acid profiles into the *Rhodococcus fascians* cluster. A recent study of Sinha et al. (2017) revealed a seasonal dominance of *R. fascians* in Arctic fjord heterotrophic bacterial communities. Phylogenetic analysis of 16S rRNA genes from alkane-degrading bacterial isolates indicated that *Rhodococcus* spp. from cold regions mostly group with *R. erythropolis* or *R. fascians* (Aislabie et al. 2006). We have isolated a large number of alkanotrophic *Rhodococcus* strains identified as *R. erythropolis*, *R. fascians*, *R. opacus*, *R. rhodochrous*, and *R. ruber* from soil, surface and spring water, snow, air, and core samples taken from ecologically and geographically diverse regions of the former Soviet Union (Ivshina et al. 1994, 1995).

However, most of the recent environmental microbiology studies using molecular genetic techniques focused on bacterial community structure and dynamics rather than on culture isolation. The occurrence of four alkane monooxygenase genotypes (*Pseudomonas putida* GPo1, Pp *alkB*; *Rhodococcus* sp. strain Q15, Rh *alkB1* and Rh *alkB2*; and *Acinetobacter* sp. strain ADP-1, Ac *alkM*) in hydrocarbon-contaminated and pristine soils from the Arctic and Antarctica was determined by both culture-independent (PCR hybridization analyses) and culture-dependent (colony hybridization analyses) molecular methods (Whyte et al. 2002a). PCR hybridization of the

total soil community DNA revealed that Rh *alkB1* and Rh *alkB2* genes are common in both contaminated and clean soils, whereas Pp *alkB* is common in contaminated soil, while Ac *alkM* is rare. Furthermore, Rh *alkB1* was prevalent in culturable psychrotolerant bacteria, suggesting that *Rhodococcus* is the predominant alkane degrader in both pristine and contaminated polar soils. Similar results for Rh *alkB1* and Rh *alkB2* prevalence in polar (Antarctica), Alpine (Austria), and tropical (Brazil) soils (Margesin et al. 2003; Luz et al. 2004) suggested that rhodococci are typical alkanotrophic soil bacteria through various ecological and climatic regions. However, in a recent study of Nie et al. (2014), only a few *alkB* sequences retrieved from freshwater, marine, and terrestrial metagenomic databases were closely related to the sequences found in previously identified microbial genomes, suggesting the presence of numerous novel *alkB* genes, including those from *Rhodococcus*, in different environments. Since rhodococci are known to harbor multiple *alkB* genes for different alkane 1-monooxygenases, similarity levels of *alkB* genes with the unique nucleotide sequence encoding a conserved amino acid motif:WLG(I/V/L)D(G/D)GL can be useful for revealing the evolution and improving systematic of this genus (Táncsics et al. 2015).

Phylogenetic comparison of pristine and hydrocarbon-contaminated Alpine soils using DGGE fingerprinting of PCR-amplified 16S rRNA gene sequences indicated the abundance of the *Actinobacteria* phylum members, *Rhodococcus* and *Mycobacterium* (Labbé et al. 2007). Microbial communities of heavy fuel-impacted shoreline in north Spain analyzed by DGGE of PCR-amplified 16S rDNA also contained high proportions of *Rhodococcus* members associated with weathered and biotreated contaminations, suggesting that this genus may be important for biodegradation of high-molecular-weight hydrocarbons (Jiménez et al. 2007).

2.2 *Rhodococci Can Be Successfully Enriched in Laboratory Hydrocarbon-Oxidizing Consortia*

There are considerable numbers of studies showing that rhodococci play a leading role in aerobic biodegradation of mono- and polyaromatic compounds, highly toxic to many bacterial species (for a review, see Martínková et al. 2009). Taki et al. (2007) found that in pristine and trichloroethylene-contaminated soils incubated with *o*-xylene (the most recalcitrant isomer of xylenes) and mineral nutrients, the *Rhodococcus opacus* was abundant, increasing by almost two orders of magnitude during an active *o*-xylene biodegradation as it was estimated by competitive PCR using a primer set specific for *R. opacus* and *R. koreensis*. These authors also isolated *o*-xylene-degrading *Rhodococcus* strains that may be effective in the bioaugmentation of soil polluted with BTEX (benzene, toluene, ethylbenzene, and xylene). A *Rhodococcus* sp. isolated from PAH-contaminated river sediment utilized anthracene, phenanthrene, pyrene, and fluoranthene as a sole source of carbon and energy (Dean-Ross et al. 2001). Fahy et al. (2008a) have investigated two

groundwater samples from a BTEX-contaminated aquifer located below a petrochemical plant using 16S rDNA fingerprinting and found that aerobic benzene-degrading communities contain *Actinobacteria*, including *Rhodococcus* and *Arthrobacter*, which were enriched at high benzene concentrations. Alkali-tolerant benzene-degrading *R. erythropolis* strains were isolated; they have potential applications in bioremediation or natural attenuation of aromatic-contaminated alkaline waters (Fahy et al. 2008b). The prevalence of *Rhodococcus* in benzene- and toluene-degrading bacterial communities of compost-based biofilters treating air polluted with aromatic compounds was confirmed by cultivation-dependent (plate counts and isolated strain identification) and cultivation-independent [automated ribosomal intergenic spacer analysis (ARISA) and PCR-DGGE of 16s rRNA gene] methods (Juteau et al. 1999; Borin et al. 2006). It was hypothesized that *Rhodococcus* cells originally present in compost at very low concentrations were enriched during biofilter operation at high benzene/toluene load and they have outcompeted other aromatic-degrading bacteria, such as *Pseudomonas*.

Soils, sediments, and waters contaminated with chlorinated hydrocarbons, nitroaromatic and complex heterocyclic compounds could also be a source for isolation of metabolically active *Rhodococcus* strains (Coleman et al. 1998; Wagner-Döbler et al. 1998; Poelarends et al. 2000; Seth-Smith et al. 2002; Petrić et al. 2007; Ito et al. 2016). For example, in the biphenyl-enriched microbial communities isolated from soils and sediments contaminated with PCBs, the majority of strains was identified as *R. opacus*, which outcompeted other biphenyl-mineralizing bacteria in the microcosms during long-term enrichment, thereby demonstrating a great potential for use in bioremediation requiring long-term survival of inocula (e.g., for recalcitrant xenobiotic compounds, such as PCBs) (Wagner-Döbler et al. 1998). In a similar study of Petrić et al. (2007), the isolated *R. erythropolis* strain was characterized by the highest PCB-transformation potential comparable with that of the parental mixed culture obtained from PCB-contaminated soil. Several *Rhodococcus* strains able to aerobically degrade 2,4,6-trinitrophenol and hexahydro-1,3,5-trinitro-1,3,5-triazine were isolated from soils heavily contaminated with nitrophenols and explosives (Coleman et al. 1998; Seth-Smith et al. 2002; Shen et al. 2009b). An endosulfan sulfate-degrading *R. koreensis* strain S1-1 with a new metabolic pathway was isolated from soil long-term contaminated with this pesticide (Ito et al. 2016).

A large body of research showed that rhodococci are abundant and often predominant components of natural and industrial biofilms developing upon the contact with hydrocarbon contamination (Sorkhoh et al. 1995; Tresse et al. 2002; Di Lorenzo et al. 2005). For example, *Rhodococcus* members were most abundant among indigenous oil-degrading bacteria immobilized in cyanobacterial mats on crude oil-contaminated coasts of the Arabian Gulf (Sorkhoh et al. 1995). Microbial consortia of artificially developed oil-degrading biofilms on gravel particles and glass plates included hydrocarbon-oxidizing bacteria, namely, nocardioforms (a group to which rhodococci belong) and *Acinetobacter calcoaceticus* partly attached to filaments of cyanobacteria (Al-Awadhi et al. 2003). The authors discussed a potential use of these biofilms for preparing trickling filters (gravel particles) and in bioreactors (glass plates) for biotreatment of oily wastes and oil-

contaminated waters. Complex toluene-degrading biofilms developed on pumice granules in the laboratory bioreactor, following the inoculation with a microbial consortium obtained by enrichment of toluene-contaminated water (Di Lorenzo et al. 2005). Interestingly, the identification of the species present in the biofilm based on 16S rDNA comparative analysis revealed that the majority (85%) of the attached cells was represented by *R. erythropolis*, whereas *Pseudomonas marginalis* represented only 10% of the entire consortium. On the contrary, in the inoculum used for biofilm development, *P. marginalis* was predominant (86%), and *R. erythropolis* was only 10% of the consortium. Apparently, adhesion to the pumice support promoted the growth of *R. erythropolis*, modifying the initial ratio between the two species. Masy et al. (2016a) demonstrated *R. erythropolis* biofilm formation on gravel and such biofilm was stable during 15-month experiment in real car parking conditions and it was able to limit hydrocarbon leaching from artificial rainfall.

From the results of numerous studies referenced above on the occurrence and frequent dominance of *Rhodococcus* members in diverse pristine and human-impacted ecosystems, it is clear that this genus is ubiquitous in the environment, associating with the presence of hydrocarbons and their substituted derivatives. There are increasing numbers of *Rhodococcus* strains isolated from clean and contaminated environments able to degrade/transform hydrocarbon contaminants and xenobiotics; these strains are available from the culture collections worldwide (Home Pages of Culture Collections in the World 2018).

2.3 *Outstanding Physiological, Biochemical, and Ecological Properties of* Rhodococcus

Physiologo-biochemical and ecological properties of *Rhodococcus* suitable for environment bioremediation are listed in Table 1. First, rhodococci are characterized by high catabolic diversity and unique enzymatic capabilities. Chemical pollutants degraded by rhodococci range from aliphatic and aromatic hydrocarbons through chlorinated hydrocarbons and nitroaromatics to complex poly- and heterocyclic compounds. The reader is referred to the fundamental review of Warhurst and Fewson (1994) and more recent reviews (van der Geize and Dijkhuizen 2004; Larkin et al. 2005; Martínková et al. 2009). A number of studies on the degradation of most abundant environmental contaminants, crude oil and its refinery products, by rhodococci have been carried out (Whyte et al. 1998, 2001; Sharma and Pant 2000), and although many other bacteria can also degrade petroleum hydrocarbons, some novel catalytic pathways have been described in *Rhodococcus* (Whyte et al. 2002a, b; Kim et al. 2004; van der Geize and Dijkhuizen 2004). Metabolic studies were focused on the rhodococcal pathways for alkane, cycloalkane, mono- and polyaromatic hydrocarbon biodegradation (Dean-Ross et al. 2001; Kim et al. 2004; Whyte et al. 2002a, b; Larkin et al. 2005; Lee and Cho 2008), transformations of nitrogen and sulfur compounds (Xu et al. 2006; Shen et al. 2009a, b), and the

Table 1 Physiological, biochemical, and ecological properties of *Rhodococcus* suitable for environmental bioremediation

Characteristics	Description	Advantage in bioremediation	References
High catabolic diversity	Degradation of wide range of chemicals, including aliphatic and aromatic hydrocarbons and their nitro- and halogenated derivatives, oxygenates, and heterocyclic compounds	Biodegradation of complex contaminations, e.g., crude oil and industrial wastes. Biodegradation of recalcitrant xenobiotics	Warhurst and Fewson (1994), van der Geize and Dijkhuizen (2004), and Martínková et al. (2009)
Aerobic and microaerophilic metabolism	Persistence in low-oxygen environments and activation upon oxygen supply	Bioremediation under well-aerated and oxygen-limited conditions (e.g., in high-density soils, sediments and hypoxic aquifers)	Travkin et al. (2002), Fahy et al. (2006, 2008a), Vogt et al. (2004), and Joshi et al. (2008)
Oligotrophy and nitrogen fixation	Growth under nutrient limitation conditions (C/N limitation) and resistance to long-term starvation	Bioremediation of resource-limited environments, e.g., low-nutrient soils and groundwaters	Ivshina et al. (1981), Elo et al. (2000), Mergaert et al. (2001), Priestley et al. (2006), and Ohhata et al. (2007)
Lack of catabolic repression	Biodegradation of contaminants is not repressed by the presence of easily assimilable nutrients	Bioremediation of organic-rich environments, e.g., wastewaters	Warhurst and Fewson (1994)
Adaptation to hydrophobic substrates	Cell surface hydrophobicity, adhesion to hydrocarbons, and biosurfactant production	Enhanced biodegradation of hydrophobic pollutants	Lang and Philp (1998) and Whyte et al. (1999)
Adhesion and biofilm formation	Adhesion to solid surfaces, cell aggregation, and biofilm formation	Application as immobilized or self-immobilized biocatalysts	Sorkhoh et al. (1995), Di Lorenzo et al. (2005), and Borin et al. (2006)
Resistance to environmental stresses	Psychrotrophy, thermo-, xero-, and galotolerance, pH-resistance, resistance to heavy metals and xenobiotics	Bioremediation in extreme environments and harsh industrial conditions	Whyte et al. (1999), Bej et al. (2000), Aislabie et al. (2006), Ryu et al. (2006), and Fahy et al. (2008b)
Ecological behavior	*K*-strategy and high competitiveness in communities under crowded, substrate-limited conditions	Survival in bioaugmentation inoculaand indigenous communities	Juteau et al. (1999), Margesin et al. (2003), and Borin et al. (2006)

(continued)

Table 1 (continued)

Characteristics	Description	Advantage in bioremediation	References
Nonpathogenicity and lack of antagonistic properties	Low number of pathogenic species (only *R. equi* and *R. fascians*), lack of antimicrobial activity, plant biosafing	Biosafety and environmental safety	Bell et al. (1998), Nagy et al. (1995), Uroz et al. (2003), and Aoshima et al. (2007)

interactions between rhodococci and other oil degraders (Van Hamme et al. 2003; Hamamura et al. 2006). Chlorophenols, chlorobenzenes, and polychlorinated biphenyls can be degraded by rhodococci (for review see Martínková et al. 2009), as well as other recalcitrant and toxic pollutants including nitriles (Baxter et al. 2006), sulfonated azo dyes (Joshi et al. 2008), pesticides (Nagy et al. 1995; Ito et al. 2016), pharmaceuticals (Ivshina et al. 2015b; Homklin et al. 2012), cyanides (Baxter and Cummings 2006; An et al. 2018), benzothiazoles (Besse et al. 2001), hydrofurans (Daye et al. 2003; Tajima et al. 2012), and many others.

2.3.1 Adaptation to Hydrocarbon Assimilation

Apart from remarkable biodegradative abilities, rhodococcal adaptation to hydrophobic contaminants is the important matter for bioremediation (Pieper and Reineke 2000; Ivshina et al. 2017). It is assumed that prolonged persistence of hydrophobic organic compounds in the environment is mainly determined by their solubilization-limited bioavailability for microorganisms. Many bacteria can assimilate hydrophobic substances, such as hydrocarbons, only in solubilized or emulsified forms. On the contrary, the hydrocarbon uptake by *Rhodococcus* occurs via the direct cell contact with large oil drops (Lang and Philp 1998). Thus, Whyte et al. (1999) observed physiological adaptations involved in alkane assimilation by *Rhodococcus* cells at a low temperature; these included production of cell-bound biosurfactants, increase in cell surface hydrophobicity, production of intracellular inclusions and extracellular polymers, and alteration of membrane fluidity. The authors suggested that high cell hydrophobicity and cell-associated biosurfactants promote the adhesion of rhodococcal cells not only to liquid alkanes but also to hydrophobic solid surfaces, allowing direct uptake from sorbed/crystalline hydrocarbons, another important mechanism of microbial assimilation of hydrophobic pollutants in soils and sediments (Wattiau 2002). In our experiments, *Rhodococcus* biosurfactants desorbed crude oil and PAHs from soil, thus facilitating its biodegradation by soil microorganisms (Kuyukina et al. 2005; Ivshina et al. 2016). It should be noted that many hydrocarbons, for example, low-molecular-weight alkanes, monoaromatics, and chlorinated aliphatic compounds, are toxic to microorganisms primarily due to the solvent effect on cell membranes. They destroy microbial cells and therefore abolish the desired biodegradative activity. Several *Rhodococcus* strains resistant to organic

solvents have been isolated, and possible mechanisms of solvent tolerance, such as alterations in the composition of cell envelope, have been reported (see chapter "Adaptation of *Rhodococcus* to Organic Solvents" by de Carvalho).

2.3.2 Ecological Plasticity

Another important *Rhodococcus* feature is the ability to persist and metabolize in microaerophilic and oligotrophic conditions. Rhodococci could be isolated from microaerophilic environments, for example, high-density soils, deep-sea sediments, and hypoxic aquifers (Colquhoun et al. 1998; Hendrickx et al. 2005; Fahy et al. 2006). The study of Travkin et al. (2002) reported the isolation of an enrichment culture and a *Rhodococcus* strain derived from it, transforming 3,4-halogenated anilines under nitrate-reducing conditions. Anaerobic bioconversion of these haloanilines by *Rhodococcus* sp. started with reductive deamination, resulting in production of dihalobenzene intermediates, which were further dehalogenated in the biodegradation pathway. A natural bacterial consortium consisting of *Aeromonas caviae*, *Proteus mirabilis*, and *R. globerulus* was reported to decolorize azo dyes under microaerophilic condition in the presence of the organic carbon source (Joshi et al. 2008). The consortium decolorized 14 azo dyes individually as well as in simulated mixed wastewater, suggesting its possible application in industrial wastewater treatment. Although the abovementioned studies reveal the possibility of *Rhodococcus* application in microaerophilic/anaerobic biodegradation of contaminants, the vast majority of extensive researches resume that rhodococci can persist well in low-oxygen environments and rapidly increase in number upon the oxygen supply, thus contributing to aerobic (oxidative) environmental decontamination (Vogt et al. 2004; Fahy et al. 2008a). Ohhata et al. (2007) isolated a *R. erythropolis* strain N9T-4 from a crude oil sample and found that this strain and some other collection *R. erythropolis* strains grew in extremely oligotrophic conditions, suggesting that the oligotrophy could be a common feature of *Rhodococcus* (Mergaert et al. 2001). Additionally, there are several reports on the abilities of rhodococci to grow and degrade organic contaminants under carbon- and nitrogen-limiting conditions (Priestley et al. 2006), to fix atmospheric nitrogen (Ivshina et al. 1981; Elo et al. 2000), and to oxidize complex pollutants even in the presence of more easily assimilable carbon sources (Warhurst and Fewson 1994). These features make rhodococci the promising candidates for bioremediation of both resource-limited and organic-rich environments.

Many contaminated sites are characterized by harsh environmental conditions, for example, low or elevated temperatures, acidic or alkaline pH, high salt concentrations, or high pressure. Apparently, extremotolerant *Rhodococcus* members adapted to grow and thrive in these environments play an important role in bioremediation of polluted extreme habitats (Sorkhoh et al. 1990; Whyte et al. 1999; Aislabie et al. 2006; Ryu et al. 2006; Fahy et al. 2008b). A hydrocarbon-degrading potential of cold-adapted rhodococci was mentioned earlier in this chapter. It is assumed that low temperature greatly influences the process of hydrocarbon

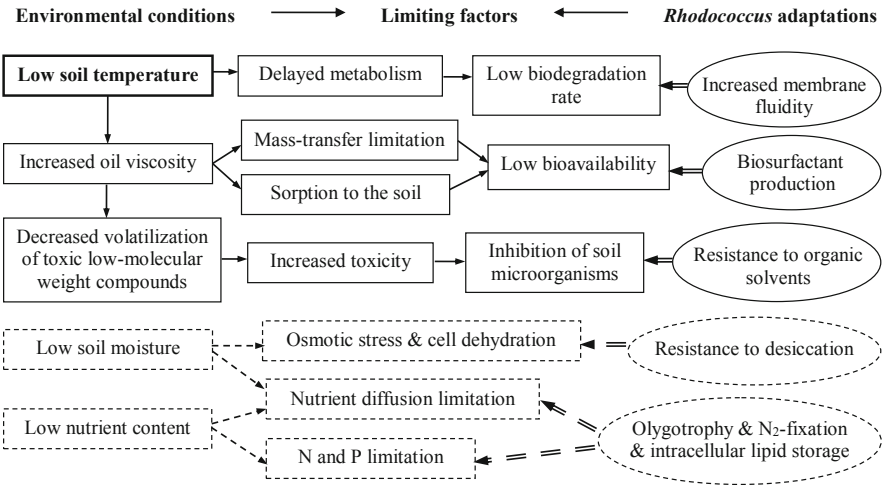

Fig. 2 Adaptations of *Rhodococcus* to cold soil conditions. Optional environmental conditions, factors, and corresponding adaptations are shown by dotted lines

biodegradation in soil by affecting both the physical nature of spilled oil and microbial metabolism (Fig. 2). Particularly, at low temperatures, the oil viscosity is increased, and the volatilization of toxic low-molecular-weight compounds is reduced, thus decreasing the bioavailability and biodegradation of hydrocarbon pollutants. Additionally, soils of cold regions are often subject to drying because of low precipitation and long freezing periods. The low soil moisture results in nutrient diffusion limitations and, in addition to typically low available N and P contents of these soils, leads to development of oligotrophic conditions for soil microorganisms (Aislabie et al. 2006). On the one hand, it is important that complex physiological adaptations of rhodococci (see Fig. 2) allow them to survive in such extreme cold environments as well as to contribute to microbiological degradation of hydrophobic pollutants (Bej et al. 2000). On the other hand, indigenous mesophilic hydrocarbon-oxidizing rhodococci were suggested as promising bioremediation agents for hot climate regions, for example, for the Kuwaiti desert soil heavily contaminated with crude oil (Sorkhoh et al. 1990). Moreover, there are increasing numbers of reports on the isolation of heat-resistant enzymes, such as specific dioxygenases, catalyzing initial steps of degradation of (poly)aromatic compounds by *Rhodococcus* cells (Gakhar et al. 2005; Yang et al. 2008). These enzymes, due to their high chemical and thermal stability, offer powerful tools for biological treatment of polluted environments and industrial wastes at elevated temperatures. One more important aspect of bioremediation is rhodococcal resistance to heavy metal ions and their bioaccumulation, including those that are radioactive (Bell et al. 1998; Ivshina et al. 2002, 2013). Since heavy metals are often present in hydrocarbon-contaminated soils associated with oil spills, petrochemical and other chemical waste discharges, and in industrial effluents, rhodococci can be used for bioremediation of such complex metallo-organic contaminations (Kuyukina et al. 2017).

The ability of rhodococci to adhere to different surfaces and to form biofilms is widely used to develop immobilized biocatalysts suitable for various eco-biotechnological applications, namely, bioreactor treatment of contaminated waters and sludges, and soil bioremediation (Prieto et al. 2002a; Di Lorenzo et al. 2005; Podorozhko et al. 2008; Kuyukina et al. 2009, 2017; Hatzinger et al. 2017). It is assumed that microorganisms in biofilms are more resistant to environmental stressors than in a free-living state. Therefore, rhodococci with high adhesive activities, especially toward hydrophobic surfaces, tend to be more successful in colonizing of hydrocarbon-contaminated sites compared to less adhering microorganisms (Masák et al. 2004). Relative prevalence of *Rhodococcus* species in many natural and laboratory contaminant-degrading microbial consortia may be also explained by their ecological behavior, particularly the *r-K* scheme, which suggests that evolution favors either adaptation to high rates of reproduction (*r* strategists) or optimal utilization of environmental resources (*K* strategists) (Margesin et al. 2003). Bacteria, such as pseudomonads, which rapidly grow in nutrient-rich media, are *r* strategists. Others, such as rhodococci, tend to be more successful in resource-limited, crowded environments, are *K* strategists. Apparently, populations of *K* strategists would be more stable and permanent members of the communities of chronically contaminated biotopes or bioreactor microcosms, when easy-degradable substrates are depleted and the competition for nutrients is intensive.

2.3.3 Biosafety Aspects

One potential problem with bioaugmentation is that the inocula may contain microorganisms harmful for the human health or environment, for example, human, animal, and plant pathogens or strains producing toxins or antibiotics, which were present in indigenous populations or came from laboratory media contamination and were enriched during the inoculum growth. To prevent the pathogen occurrence in bioaugmentation inocula, such consortia should contain only taxonomically defined microorganisms belonging to species known as nonpathogenic. Moreover, in some countries, national environmental and health authorities require an assessment of biosafety and environment safety of microbial inocula intended for bioremediation applications (Aoshima et al. 2007). The study of Aoshima et al. (2007) evaluated the safety of the hydrocarbon-oxidizing soil isolate *R. erythropolis* C2 for the application in open oil-contaminated ecosystems and found that this strain demonstrates the lack of pathogenicity, mutagenicity, or ecotoxicity. It therefore requires no special occupational health precautions during application processes and has low environmental impact. These results are in agreement with other literature data indicating that only two *Rhodococcus* species, *R. equi* and *R. fascians*, are associated with pathogenicity for animals and plants, respectively (chapters "Genetics of the Virulence of *Rhodococcus equi*" by Vázquez-Boland and "Phytopathogenic Strategies of *Rhodococcus fascians*" by Vereecke). Such relatively small proportion of pathogenic species is rare within the micolata group of actinobacteria harboring genera *Corynebacterium*, *Gordonia*, *Mycobacterium*, *Nocardia*, and *Tsukamurella*,

characterized by abundant presence of human pathogens. Additionally, literature and our research data suggest that rhodococci are unlikely to produce any toxins or antimicrobial compounds (Kitamoto et al. 2002; Kuyukina et al. 2007); this is another strong advantage of *Rhodococcus* applications in environment bioremediation technologies. There are only two reports by Kitagawa and Tamura (2008a, b) on *R. erythropolis* producing antibiotics active against Gram-positive bacteria, including *Rhodococcus* and related genera. Furthermore, the rhizospheric *R. erythropolis* strain W2 degrading *N*-acylhomoserine lactones was shown to be effective in quenching of quorum-sensing-regulated functions of plant pathogenic bacteria, thereby reducing their pathogenicity (Uroz et al. 2003). This bacterium and herbicide-degrading *Rhodococcus* strains applicable for plant biosafing (Nagy et al. 1995; Hongming et al. 2015) could be used in phytoremediation projects for contaminated agricultural soils. Additionally, Shagol et al. (2014) described an arsenic-tolerant plant-growth-promoting *R. aetherivorans* strain isolated from smelter-polluted soil, which consistently increased root length of maize in the presence of 100 and 200 μM As(V) and can therefore enhance the efficiency of phytoremediation in As-polluted soils.

3 *Rhodococcus* Applications in Bioremediation Technologies

Biological remediation of terrestrial and aquatic habitats contaminated with hazardous compounds received increasing attention in early 1990s, with enhanced awareness of the potential harmful effects on human health and the environment (Alexander 1999). Various bioremediation techniques have been used at a large number of sites contaminated with organic pollutants since the most well-known cleanup of oil spilt from the Exxon Valdez in Prince William Sound, Alaska, in 1989 (Van Hamme et al. 2003). A historical aspect of *Rhodococcus* application in environment bioremediation could be addressed to late 1990s, when first attempts were made to remediate oil-contaminated lands using naturally accruing rhodococci or laboratory *Rhodococcus* cultures (Sorkhoh et al. 1995; Koronelli et al. 1997; Christofi et al. 1998). Upon revealing new catabolic abilities of *Rhodococcus* species and isolation of environmental strains degrading a wide range of contaminants, these bacteria have been increasingly explored for bioremediation of soils, waters, and air polluted with different recalcitrant and toxic organic chemicals (Fig. 3).

3.1 In Situ Treatment

In situ bioremediation comprises various techniques, which treat contaminated material in place (without excavation and transfer) and keep the material treated

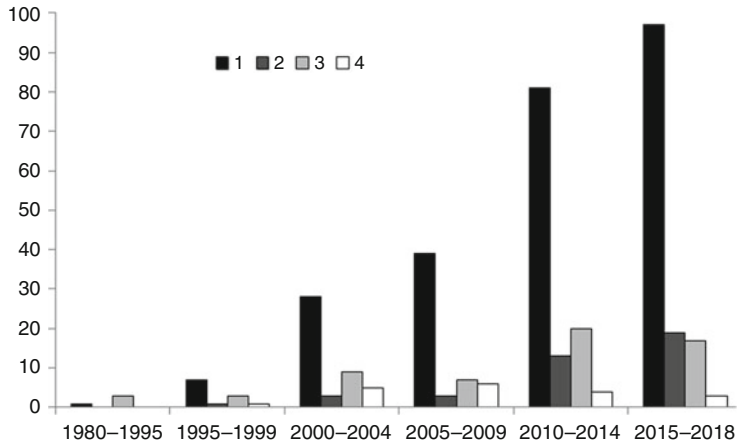

Fig. 3 Number of research articles concerned to *Rhodococcus* applications in environment bioremediation (according to http://www.scopus.com). Queries: Title/Abstract/Keywords: *Rhodococcus* and (1) soil bioremediation; (2) groundwater bioremediation; (3) bioreactor degradation; (4) air biofilter. Nonrelevant papers were removed from the query results

essentially undisturbed. These techniques are usually applied to the remote or difficult of access polluted environments, such as forest soils and wetlands, aquatic sediments, and subsurface zones, as well as in cases when intrusive methods are inappropriate, for example, for remediation of unique landscapes and valued soils. Most in situ processes involve stimulation of indigenous microbial populations through the addition of nutrients and other factors enhancing biodegradation (e.g., surfactants and oxygen source) and sometimes the augmentation with specifically adapted microbial cultures possessing high biodegradative abilities (Van Hamme et al. 2003). The limited number of laboratory and field studies referenced in Table 2 suggested that rhodococci, either as parts of bioaugmentation inocula or members of indigenous communities, can be successfully used for reducing in situ organic contaminant levels. Thus, laboratory soil microcosm study using an indigenous psychrotolerant alkane-degrading *Rhodococcus* strain ADH reported a positive effect of bioaugmentation on the biodegradation of diesel fuel (Ruberto et al. 2005). Although the natural microflora responded significantly to the pollutants, *Rhodococcus*-inoculated microcosms showed enhanced biodegradation compared to non-inoculated soil and sterilized controls. The survival and activity of *Rhodococcus* sp. strain 1BN introduced into naphthalene-contaminated sandy-loam soil were studied by Cavalca et al. (2002). The naphthalene consumption and CO_2 production rates were the highest in the *Rhodococcus*-amended sterilized soil, although inoculation of non-sterile soil did not enhance significantly the biodegradation process, indicating a considerable bioremediation potential of the indigenous naphthalene-degrading bacteriocenosis. Nevertheless, the introduced *Rhodococcus* strain was well-established in the contaminated soil even in the presence of native naphthalene-degrading bacteria. In our experiments, poly(vinyl alcohol) cryogel-immobilized *R. ruber* and *R. erythropolis* survived successively in oil-contaminated

Table 2 Selected examples of *Rhodococcus* application for bioremediation of contaminated environments

Contaminated substrate	Bioremediation method	*Rhodococcus* application mode	Effect of treatment	References
In situ treatment—treatability studies				
Antarctic soil contaminated with diesel fuel	Laboratory bioaugmented soil system exposed to natural climate conditions of Antarctica	Liquid culture of *Rhodococcus* sp.	+	Ruberto et al. (2005)
Naphthalene-contaminated soil from industrial area	Laboratory bioaugmented soil systems	Liquid culture of *Rhodococcus* sp.	+	Cavalca et al. (2002)
PAH-contaminated soil from petroleum refinery site	Laboratory bioaugmented soil systems	Liquid co-culture of *Rhodococcus* sp. and *Aspergillus terreus*/*Penicillium* sp.	+	Kim and Lee (2007)
Diesel-contaminated soil	Soil inoculated with diesel-degrading rhizobacterium and seeded with diesel-resistance *Zea mays*	Liquid culture of *Rhodococcus* sp.	+	Hong et al. (2007)
Soil contaminated with crude oil	Laboratory systems with preheated and non-heated soils	Poly(vinyl alcohol) cryogel-immobilized culture of *R. ruber* and *R. erythropolis*	+	Kuyukina et al. (2013)
Aroclor 1242-contaminated soil	Laboratory soil column with mineral nutrient addition, bioaugmented with bacteria and earthworms	Liquid culture of *Ralstonia eutrophus* and *Rhodococcus* sp.	+	Luepromchai et al. (2002)
Aroclor 1242-contaminated sediment	Laboratory two-stage anaerobic/aerobic biotreatment (aerobic stage)	Liquid culture of recombinant *Burkholderia xenovorans* and *Rhodococcus* sp.	+	Rodrigues et al. (2006)
Groundwater artificially contaminated with hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)	Repacked aquifer sediment columns operated at 15 °C	Liquid culture of *Gordonia* sp., *Pseudomonas fluorescens*, and *R. jostii*	+	Fuller et al. (2015)
Tap water artificially contaminated with gasoline	Laboratory aerobic biobarriers filled with volcanic pumice	Liquid culture of *Rhodococcus* sp. and *Methylibium petroleiphilum*	±[a]	Daghio et al. (2015)

(continued)

Table 2 (continued)

Contaminated substrate	Bioremediation method	*Rhodococcus* application mode	Effect of treatment	References
Benzene-contaminated groundwater	Laboratory groundwater microcosms	Indigenous bacterial community containing *Rhodococcus*	+	Fahy et al. (2006, 2008a)
In situ treatment—field studies				
Shoreline contaminated with heavy fuel oil	Plots on the cobblestone beach spread with oleophilic fertilizer S200 (IEP Europe)	Indigenous bacterial community containing *Rhodococcus*	+	Jiménez et al. (2007)
Crude oil-polluted river bay, lakes, wetland, and marshy peat soil	Preliminary mechanical collection of spilled oil using skimmers followed by spraying with biopreparation and mineral nutrients	Liquid biopreparation *Rhoder* consisting of *R. erythropolis* and *R. ruber*	+	Murygina et al. (2000)
Groundwater polluted with methyl *t*-butyl ether and *t*-butyl alcohol	Biobarrier plots with O_2 or air sparging	Mixed culture or pure *Rhodococcus* sp. culture isolated from polluted groundwater	+	Salanitro et al. (2001)
Chlorobenzene-polluted groundwater	Reactive barrier supplied with hydrogen peroxide and nitrate solution	Indigenous bacterial community containing *Rhodococcus*	+	Vogt et al. (2004)
Petroleum hydrocarbon polluted groundwater	Closed bipolar system (one extraction and two injection wells)	Pre-grown consortium of zymogenous microorganisms containing *Rhodococcus*	+	Beškoski et al. (2017)
On-site treatment—treatability studies				
Crude oil-contaminated Arctic soil spiked with *n*-hexadecane	Laboratory bioaugmented soil systems	Liquid culture of *Rhodococcus* sp.	+	Whyte et al. (1998)
Arctic soil contaminated with *n*-alkanes or diesel fuel	Laboratory soil systems with additions of inorganic/organic fertilizers and bulking agents	Indigenous bacterial community containing *Rhodococcus*	+	Whyte et al. (2001)
Arctic soil contaminated with weathered diesel fuel	Laboratory bioaugmented soil system	Liquid enriched indigenous culture containing *Rhodococcus*	±[a]	Thomassin-Lacroix et al. (2002)

Soil contaminated with crude oil	Laboratory soil systems with mineral nutrient addition	Indigenous bacterial community containing *Rhodococcus*	+	Peressutti et al. (2003)
Soils from geographically distinct areas contaminated with weathered crude oil	Laboratory soil systems with mineral nutrient addition	Indigenous bacterial community containing *Rhodococcus*	+	Hamamura et al. (2006)
Soil contaminated with fuel oil	Laboratory bioaugmented soil plots with bulking agent and mineral nutrient addition	Liquid biopreparation *Devoroil* containing *R. erythropolis* and *Rhodococcus* sp.	+	Sidorov et al. (1998)
Soil contaminated with BTEX	Laboratory bioaugmented soil systems with mineral nutrient addition	Liquid culture of *R. opacus*	+	Taki et al. (2004)
Soil contaminated with BTEX	Laboratory soil systems with mineral nutrient addition	Indigenous bacterial community with dominating *Rhodococcus*	+	Taki et al. (2007)
Soil contaminated with disulfide oil	Laboratory bioaugmented soil systems with mineral nutrient and glucose addition	Vermiculite-immobilized culture of *Rhodococcus* sp.	+	Taheri et al. (2008)
On-site treatment—field studies				
Crude oil-contaminated desert soil	Bioaugmented landfarming cells with addition of mineral nutrients and lime	Liquid mixed culture containing *Rhodococcus*, removed from cyanobacterial mats floating in oil-polluted waters	+	Sorkhoh et al. (1995)
Tundra soil artificially contaminated with crude oil	Bioaugmented soil plots with mineral nutrient addition	Lyophilized culture of *R. erythropolis*	+	Koronelli et al. (1997)
Soil artificially contaminated with crude oil and oil-field brine	Bioaugmented landfarming cells with mineral nutrient addition	Liquid biopreparation *Devoroil* containing *R. erythropolis* and *Rhodococcus* sp.	+	Sidorov et al. (1997)
Crude oil-contaminated soil	Composted inoculated biopiles with addition of mineral nutrients and straw as bulking agent	Liquid culture of biosurfactant-producing *R. ruber*	+	Christofi et al. (1998)
Arctic soil contaminated with weathered diesel fuel	Inoculated biopiles with addition of fertilizer (granular urea and diammonium phosphate), surfactant, and cocoa-fiber bulking agent	Liquid enriched indigenous culture containing *Rhodococcus*	±[a]	Thomassin-Lacroix et al. (2002)

(continued)

Table 2 (continued)

Contaminated substrate	Bioremediation method	*Rhodococcus* application mode	Effect of treatment	References
Crude oil-contaminated soil	Landfarming cells with addition of woodchips and oleophilic biofertilizer	Oleophilic biofertilizer containing *R. erythropolis* and *R. ruber*	+	Kuyukina et al. (2003)
Polar marshy wetland polluted with crude oil	Landfarming + phytoremediation with addition of biopreparation, inorganic fertilizer, and lime	Liquid biopreparation *Rhoder* consisting of *R. erythropolis* and *R. ruber*	+	Murygina et al. (2005)
Dehydrated oil sludge	Inoculated biopiles with addition of sand, woodchips, and inorganic fertilizer	Liquid biopreparation *Rhoder* consisting of *R. erythropolis* and *R. ruber*	+	De-qing et al. (2007)
Soil contaminated with fuel hydrocarbons	Inoculated biopile with addition of $(NH_4)_2HPO_4$	Liquid cultures of *Pseudomonas putida*, *Acinetobacter johnsonii*, and *R. erythropolis*	+	Genovese et al. (2008)
Soil artificially contaminated with diesel oil and fuel oil	Inoculated biopiles with addition of rhamnolipid biosurfactant	Liquid cultures of *Gordonia alkanivorans*, *R. erythropolis*, *Acinetobacter junii*, and *Exiguobacterium aurantiacum*	+	Lin et al. (2010)
Olive mill waste (humid husk)	Pilot biopile amended with waste wool, olive leaves and twigs, wheat straw, and pigeon manure	Indigenous tannin-degrading bacterial community containing *R. rhodochrous*	+	Federici et al. (2011)
PAH-contaminated soil	On-site landfarming plots added with nutrients	Liquid culture of *R. ruber*	+	Sun et al. (2012)
Aged fuel-contaminated soil	Pilot tank mimicking groundwater flow conditions and two bioreactors	Liquid culture of *R. erythropolis*	+	Masy et al. (2016b)
Bioreactor/biofilter treatment—laboratory studies				
Soil from former coke works site contaminated with $K_2Ni(CN)_4$	Laboratory shaking-flask experiments (1 g soil/100 ml minimal salt medium) with glucose addition	Liquid culture of *Rhodococcus* sp.	±[a]	Baxter and Cummings (2006)

Soil contaminated with bis (2-ethylhexyl)phthalate	Slurry-phase batch and sequencing batch bioreactors with mineral nutrient addition	Mixed culture isolated from contaminated soil and containing *R. fascians*	+	Juneson et al. (2001)
Aged polychlorinated biphenyl-contaminated soils	Aerobic solid-phase and packed-bed loop reactors with addition of mineral nutrients, biphenyl, and methyl-β-cyclodextrins	Indigenous bacterial community containing *Rhodococcus*	+	Fava et al. (2003)
Groundnut cake naturally contaminated with aflatoxin B1	Shake-flask bioreactors	Liquid culture of *R. erythropolis*	+	Dogan et al. (2017)
Sediment contaminated with polycyclic aromatic hydrocarbons	Aerobic slurry-phase bioreactors with mineral nutrient addition	Indigenous community containing *Rhodococcus*	+	Ringelberg et al. (2001)
Phenol- and formaldehyde-contaminated synthetic and industrial wastewaters	Laboratory shaking-flask experiments with addition of phosphate, ammonium, and micronutrients	Liquid culture of *R. erythropolis*	+	Hidalgo et al. (2002a, b)
Phenol- and formaldehyde-contaminated synthetic and industrial wastewaters	Air-stirred and packed-bed column bioreactors with addition of nitrogen source and micronutrients	Biolite- and diatomaceous earth-immobilized cultures of *R. erythropolis*	+	Prieto et al. (2002a, b)
Phenol-contaminated water	Stirred-tank bioreactor	Liquid culture of *Rhodococcus* sp.	+	Yaacob et al. (2016)
Ammonium- and 2-fluorophenol-contaminated synthetic wastewater	Sequencing batch reactor	Aerobic sludge granules and liquid culture of *Rhodococcus* sp.	+	Ramos et al. (2017)
Aroclor 1242- and biphenyl-contaminated water	Granular biofilm reactor with limited aeration and addition of mineral nutrients, yeast extract, and microelements	*Rhodococcus* sp. culture immobilized on anaerobic sludge granules	±[b]	Tartakovsky et al. (2001)
Di-*n*-octyl phthalate-contaminated water	Sequencing batch reactor	Liquid cultures of *Arthrobacter* sp. and *Rhodococcus* sp.	+	Zhang et al. (2018)
1,1,2,2-Tetrachloroethane-contaminated water	Continuous stirred-tank reactor	Liquid cultures of *R. aetherivorans*	+	Cappelletti et al. (2018)
Simulated azo dye- contaminated wastewater	Laboratory flask experiments under microaerophilic condition, with addition of yeast extract and glucose	Mixed culture from dye-contaminated soil and sludge and containing *R. globerulus*	+	Joshi et al. (2008)

(continued)

Table 2 (continued)

Contaminated substrate	Bioremediation method	*Rhodococcus* application mode	Effect of treatment	References
Model petroleum- contaminated water	Fluidized-bed column bioreactor	Sawdust-immobilized culture of *R. ruber* and *R. opacus*	+	Kuyukina et al. (2009)
Oil-field wastewater	Fluidized-bed column bioreactor	Sawdust- and polyvinyl alcohol cryogel- immobilized cultures of *R. ruber* and *R. opacus*	+	Kuyukina et al. (2017) and Serebrennikova et al. (2017)
2,4-Dinitrophenol- contaminated water	Column plug-flow bioreactor	Agar granule-immobilized culture of *R. erythropolis*	+	Kitova et al. (2004)
2,4,6-Trinitrophenol or picric acid-polluted wastewater or groundwater	Sequencing batch bioreactor	Liquid culture of *R. opacus*	+	Weidhaas et al. (2007)
Artificial groundwater contaminated with *N*-nitrosodimethylamine	Propane-fed membrane bioreactor	Liquid cultures of *R. ruber*	+	Hatzinger et al. (2011)
Organic cyanide-contaminated groundwater	Fluidized-bed reactors	Coconut granular activated carbon immobilized *R. rhodochrous* and *Bacillus mojavensis*	+	An et al. (2018)
Synthetic pharmaceutical wastewater contaminated with ibuprofen and ketoprofen	Aerobic suspension-sequencing batch reactor	Indigenous agricultural community containing *R. ruber*	+	Hasan et al. (2016)
Methylhydrazine/hydrazine-contaminated wastewater	Fixed-film trickle-bed bioreactor	Coarse sand-immobilized culture of *Rhodococcus* sp.	+	Nwankwoala et al. (2001)
Artificial toluene-contaminated waste gas	Rotating biological contactor with mineral nutrient addition	Liquid culture of *Pseudomonas putida* and *R. erythropolis*	+	Vinage and von Rohr (2003)
Artificial waste air containing 2-chlorotoluene	Biotrickling filter	Polyurethane foam-immobilized culture of *Rhodococcus* sp.	+	Dobslaw and Engesser (2018)
Air polluted with benzene	Compost-packed biofilter	Indigenous compost community containing *Rhodococcus*	+	Borin et al. (2006)
Air polluted with isopropylbenzene	Two-phase partitioning bioreactor with addition of ethanol	Liquid culture of *R. erythropolis*	+	Aldric and Thonart (2008)

Volatile compounds of tobacco leaves	Polyamide fiber-packed microbioreactors	Mixed consortium of *Pseudomonas*, *Rhodococcus*, *Nocardia*, and *Micrococcus*	+	Zagustina et al. (2012)
Gas-phase mixture of methanol, α-pinene and H_2S	Biotrickling filter	Autotrophic H_2S-degrading culture, *Candida boidinii*, *R. erythropolis*, and *Ophiostoma stenoceras*	+	López et al. (2013)
Air polluted with toluene, benzo(*a*) pyrene, and formaldehyde	Vermiculite-packed column biofilter	Consortium of *R. erythropolis* and *Fusarium solani*	+	Vergara-Fernández et al. (2018)
Bioreactor/biofilter treatment—pilot/field studies				
Crude oil-contaminated soil	Field slurry-phase bioreactor with addition of oleophilic biofertilizer	Oleophilic biofertilizer containing *R. erythropolis* and *R. ruber*	+	Kuyukina et al. (2003)
Waste oil contaminated with polychlorinated biphenyls	Pilot plant consisting of UV-irradiation equipment and two successive bioreactors	Liquid culture of *Comamonas testosteroni* and *R. opacus*	+	Kimbara et al. (1998)
Industrial wastewater polluted with 1,3-dichloropropene and other chlorinated aliphatics	Pilot extractive membrane biofilm reactor	Mixed biofilm culture isolated from bioreactor and containing *R. erythropolis*	+	Katsivela et al. (1999)
Synthetic tetrahydrofuran-contaminated wastewater	Pilot membrane bioreactor	Mixed culture isolated from the industrial wastewater treatment plant and containing *R. ruber*	+	Daye et al. (2003)
Model wastewater containing 2,4,6-trinitrophenol	Pilot biological aerated filter with mineral nutrient addition	Liquid culture of *Rhodococcus* sp.	+	Shen et al. (2009a)
Industrial wastewater contaminated with hydrocarbons, surfactants, and heavy metals	Pilot biotreatment installation	Immobilized algal-bacterial co-culture containing *Rhodococcus* sp.	+	Safonova et al. (2004)
Groundwater polluted with ethyl *t*-butyl ether and gasoline	On-site pilot bioreactor	Liquid culture of *R. wratislaviensis*, *R. aetherivorans*, and *Aquincola tertiaricarbonis*	+	Fayolle-Guichard et al. (2012)

(continued)

Table 2 (continued)

Contaminated substrate	Bioremediation method	*Rhodococcus* application mode	Effect of treatment	References
Groundwater polluted with *N*-nitrosodimethylamine and *N*-nitrodimethylamine	Field-scale propane-fed fluidized-bed bioreactor	Coconut granular activated carbon immobilized *R. ruber*	+	Hatzinger et al. (2017)

[a]±, effect of bioaugmentation did not exceed that of biostimulation; [b]±, effect of *Rhodococcus* immobilized on anaerobic sewage did not exceed that of anaerobic sewage alone. *ND* not determined

soil under drought conditions, contributing to efficient alkane biodegradation (Kuyukina et al. 2013). Several treatability studies showed positive effects of *Rhodococcus* co-cultures with other bacteria and fungi, as well as rhodococcal associations with higher plants and earthworms on biodegradation of petroleum hydrocarbons and polychlorinated biphenyls in soil and sediments (Luepromchai et al. 2002; Rodrigues et al. 2006; Hong et al. 2007; Kim and Lee 2007). Additionally, in situ bioremediation can be an economically advisable and environmentally harmless approach to cleanup shorelines contaminated as a result of marine oil spills. During the field-scale trial carried out by Jiménez et al. (2007), some success in the removal of heavy fuel oil from the cobble beach on the Cantabrian coast (north Spain) polluted after the oil tanker *Prestige* crush in 2002 was achieved using the oleophilic fertilizer, and the biodegradative potential of the indigenous microbial community, including *Rhodococcus* representatives, was established.

It is assumed that engineered in situ bioremediation is a feasible and effective method for treating contaminants within the saturated zone of soil and contaminated groundwater. Engineering in situ technologies involve drilling a series of wells for direct injection of appropriate solutions into the subsurface or the construction of reactive permeable barriers allowing the passage of groundwater while promoting the biodegradation of contaminants. Although polluted subsurface and groundwater systems are often very low in oxygen and nutrients and therefore characterized by slow biological oxidation rates, several laboratory and field-scale studies described successful applications of reactive biobarriers inoculated with *Rhodococcus* pure or mixed cultures (Salanitro et al. 2001) and supplied with oxygen and mineral nutrients (Vogt et al. 2004) for degradation of recalcitrant groundwater contaminants such as chlorinated benzene, methyl *t*-butyl ether, and *t*-butyl alcohol. In a recent field study of Beškoski et al. (2017), groundwater contaminated with petroleum hydrocarbons from an underground storage tank was treated using a closed bipolar system (one extraction and two injection wells) by bioaugmentation with pre-grown indigenous hydrocarbon-degrading consortia containing *Rhodococcus* and biostimulation with nutrients. After 60 days of bioremediation, more than 95% of *n*-alkanes, terpanes, and steranes were biodegraded; phenanthrene and its methyl-, dimethyl-, and trimethyl-isomers were removed completely, suggesting the applicability of this technology for the in situ treatment of PAH-contaminated groundwater.

3.2 On-Site Treatment

As evident from Table 2, the on-site bioremediation using *Rhodococcus* mostly involves the landfarming and biopile treatment of complex petroleum hydrocarbon mixtures, including crude oil, diesel fuel, and fuel oil. Historically, landfarming was one of the first forms of on-site contamination treatment and was widely used in oil industry for the disposal and neutralization of oily wastes. The waste material is applied to clean soil and landfarming area managed by tilling, fertilization, watering, and addition of bulking agents to maintain optimum soil conditions of nutrients,

moisture, and pH. Microorganisms used in the biodegradation process are mostly indigenous soil populations. However, high concentrations of toxic contaminants present in oily wastes often hinder the development of resident oil-oxidizing microbial consortia. Therefore, the bioaugmentation with selected cultures of oil-degrading microorganisms adapted to high toxicant content and harsh environmental conditions may be used to enhance bioremediation process. It should be noted that while landfarming of oily sludges is no longer considered environmentally acceptable due to large volumes of clean soil contaminated during the process and the potential risk of contaminant leaching and emission from the treatment area (Van Hamme et al. 2003), it is still being used in many countries. More recently, ventilated and composting biopiles, which involve a greater degree of engineering and containment, have been developed for the oil-contaminated soil and sludge treatment. The contaminated material is removed to a specifically prepared area, which is usually lined with low permeability material such as high-density polyethylene or clay to minimize contaminant movement off-site. Soil biopile systems often include leachate-collecting and sometimes emission-control facilities. Construction of composting biopiles involving a succession of mesophilic and thermophilic microorganisms consists of piling the contaminated soil and mixing with an organic bulking agent such as composted agricultural waste, straw, or woodchips. The piles are aerated by either passive or forced ventilation or pile turning, and the temperature, pH, moisture, and nutrient contents are controlled.

Several laboratory studies have reported favorable effects of *Rhodococcus* augmentation on petroleum hydrocarbon biodegradation in soils at low (Whyte et al. 1998) and mesophilic (Sidorov et al. 1998; Taki et al. 2004; Taheri et al. 2008; Kuyukina et al. 2013) temperatures. Thus, mineralization of ^{14}C-labelled hexadecane at 5 °C was significantly greater in both crude oil-contaminated and pristine soil microcosms seeded with *Rhodococcus* sp. Q15 cells compared to non-inoculated control soil microcosms (Whyte et al. 1998). Moreover, efficient removal of less degradable contaminants such as aromatic hydrocarbons (including most recalcitrant *o*-xylene) and fuel oil from soil was obtained upon soil inoculation with either pure culture of *R. opacus* (Taki et al. 2004) or mixed culture of hydrocarbon-oxidizing bacteria containing *R. erythropolis* and *Rhodococcus* sp. strains (Sidorov et al. 1998). Taheri et al. (2008) performed a feasibility study for the soil polluted with disulfide oil, a waste product of liquefied petroleum gas desulfurization, and found that a vermiculite-immobilized *Rhodococcus* sp. strain previously isolated from disulfide oil-contaminated soil has a great potential for its bioremediation, although no comparison of immobilized and liquid forms of inoculum was made. There are also few reports on considerable bioremediation potential of indigenous bacterial communities inhabiting hydrocarbon-contaminated soils and containing large proportions of *Rhodococcus* representatives (Whyte et al. 2001; Hamamura et al. 2006), which could be enriched in laboratory microcosms during bioremediation process (Peressutti et al. 2003; Taki et al. 2007). However, the laboratory microcosm study of Thomassin-Lacroix et al. (2002) demonstrated low effect of bioaugmentation of fuel-contaminated Arctic tundra soil with enriched bacterial culture originated from the same soil and containing *Rhodococcus* members, indicating that biostimulation

with a mineral nitrogen source was sufficient for the soil naturally rich in hydrocarbon-degrading microorganisms (including *Rhodococcus*).

It is now generally agreed that results of field bioremediation can differ significantly from the laboratory studies due to much stronger and more complex influence of environmental factors, both abiotic and biotic, on contaminant biodegradation process. Since bench-scale feasibility results often lack representativeness to field situations, field bioremediation trials must be conducted to corroborate findings of laboratory experiments. Small-scale field experiments conducted by Koronelli et al. (1997) reported that introduction of a hydrocarbon-degrading strain of *R. erythropolis* into tundra soil artificially contaminated with crude oil resulted in increased counts of hydrocarbon-degrading bacteria and an increased rate of hydrocarbon degradation. Christofi et al. (1998) found that inoculation with biosurfactant-producing *R. ruber* increased counts of hydrocarbon-oxidizing bacteria persisted in composted crude oil-contaminated soil and enhanced oil biodegradation. More recently Sun et al. (2012) applied a liquid *R. ruber* culture for on-site landfarming of heavily PAH-contaminated soil at the abandoned cooking plant site. They found out that a combination of biostimulation and bioaugmentation significantly enhanced the removal of PAHs from the contaminated soil. Sorkhoh et al. (1995) have used naturally occurring bacterial consortia removed from cyanobacterial mats floating in oil-polluted waters in the Arabian Gulf to inoculate oil-contaminated sand. This increased removal of oil from the sand, and rhodococci appeared to predominate in microbial populations. Bacterial preparations consisting of two-component *R. erythropolis* and *R. ruber* cultures (Kuyukina et al. 2003; Murygina et al. 2005; De-qing et al. 2007); a three-component bacterial culture of *R. erythropolis*, *Pseudomonas putida*, and *Acinetobacter johnsonii* (Genovese et al. 2008); and a complex bacterial-yeast consortium of *Dietzia* (former *Rhodococcus*) *maris*, *R. erythropolis*, *Rhodococcus* sp., *Pseudomonas stutzeri*, and *Candida* sp. (Sidorov et al. 1997) were successfully used in field trials on bioremediation of soils contaminated with crude oil, fuel, and oily wastes. Masy et al. (2016b) applied electrical resistivity tomography to detect soil heterogeneities and to monitor *R. erythropolis* biodegradation activity during a pilot-scale bioremediation of aged fuel-contaminated clay loam soil. It should be noted that in most field studies referenced above, contaminated soils were seeded with bacterial inocula and amended by addition of mineral fertilizers, bulking agents, and other factors stimulating biodegradation process, thus suggesting that combination of two bioremediation approaches, bioaugmentation and biostimulation, is essential for the cleanup of hydrocarbon-contaminated soils.

3.3 Bioreactor Treatment

Bioreactors are widely used to degrade toxic compounds in industrial effluents to prevent environmental pollution. Furthermore, bioreactor treatment of contaminated soils and sludge is not yet mainstream, but growing technology that overcomes some rate-limiting and variability factors observed in landfarming and biopile processes.

Particularly, bioreactor-based technologies allow more precise control and management of biodegradation parameters such as temperature, pH, oxygen, nutrient and water contents, and homogenous distribution of contaminated material and biomass in the reactor volume, which leads to increased mass transfer and reaction rates (Van Hamme et al. 2003). Several laboratory studies reported the application of indigenous bacterial communities containing *Rhodococcus* representatives in bioreactors of different types for treatment of contaminated soils and sediments; these included solid-phase, slurry-phase, and packed-bed loop reactors and resulted in reducing the levels of phthalate esters and polychlorinated and polycyclic aromatic compounds (Juneson et al. 2001; Ringelberg et al. 2001; Fava et al. 2003). We have used an oleophilic biofertilizer in the form of concentrated emulsion of hydrocarbon-grown *R. erythropolis* and *R. ruber* cultures and mineral salt solution stabilized by a *Rhodococcus* biosurfactant for successive treatment of crude oil-contaminated soil in a field slurry bioreactor and landfarming plots and found out that high biodegradation rate for petroleum hydrocarbons can be achieved following stimulation of the degradation process in a slurry bioreactor (Kuyukina et al. 2003).

Rhodococci are also candidate organisms for use as inocula in contaminated water treatments, demonstrating promising results in laboratory simulations (see Table 2 for references). For example, inoculations with suspended and biolite/diatomaceous earth-immobilized *R. erythropolis* cells were shown to be efficient in the biotreatment of phenol- and formaldehyde-contaminated synthetic and industrial wastewaters (Hidalgo et al. 2002a, b; Prieto et al. 2002a, b). Sawdust-immobilized *R. ruber* IEGM 615 and *R. opacus* IEGM 249 cells degraded petroleum hydrocarbons (including aliphatic from C_{10} to C_{19} and polyaromatic) to a great extent when applied to a fluidized-bed bioreactor and retained high catalytic activity during repeated bioreactor cycles (Kuyukina et al. 2009, 2017). A coarse sand-immobilized *Rhodococcus* sp. culture was successfully used in a fixed-film trickle-bed bioreactor treating wastewaters contaminated with methylhydrazine/hydrazine (Nwankwoala et al. 2001). It has been reported that a mixed culture isolated from azo dye-contaminated soil and containing *R. globerulus* cells was able to decolorize azo dyes under microaerophilic conditions (Joshi et al. 2008). However, another microaerophilic biodegradation study conducted by Tartakovsky et al. (2001) showed no significant difference in reduction of Aroclor 1242 levels in *Rhodococcus*-bioaugmented and non-bioaugmented reactors, although identification of indigenous bacterial populations of the non-bioaugmented reactor by 16S rDNA sequencing revealed *Rhodococcus* members among other biphenyl-degrading bacteria. Liquid and immobilized *Rhodococcus* spp. cultures were efficiently applied to different type bioreactors treating water contaminated with phenol, di- and trinitrophenol, fluorophenol, phthalic acid ester, nitrosodimethylamine, and organic cyanide (Kitova et al. 2004; Weidhaas et al. 2007; Shen et al. 2009a; Hatzinger et al. 2011; Yaacob et al. 2016; Ramos et al. 2017; An et al. 2018; Zhang et al. 2018). Moreover, complex emergent pollutants, such as aflatoxin and pharmaceuticals, can be efficiently degraded by *Rhodococcus* in bioreactors (Hasan et al. 2016; Dogan et al. 2017). As a promising approach to mitigate biofouling in membrane bioreactors, the abovementioned quorum quenching activity of *Rhodococcus* was used

alone or in combination with chlorination, resulting in reduced levels of *N*-acyl homoserine lactones and extracellular biopolymers in the biofilm (Maqbool et al. 2015; Weerasekara et al. 2016).

Several pilot-scale bioreactor studies referenced in Table 2 involved *Rhodococcus* applications to industrial waste and wastewater treatments (Kimbara et al. 1998; Katsivela et al. 1999; Daye et al. 2003; Safonova et al. 2004; Fayolle-Guichard et al. 2012; Hatzinger et al. 2017). Particularly, pilot membrane bioreactors inoculated with *Rhodococcus*-containing mixed cultures were used to treat tetrahydrofuran and dichloropropene waste streams (Katsivela et al. 1999; Daye et al. 2003). Industrial wastewater inoculation with an algal-bacterial co-culture containing a *Rhodococcus* sp. strain Ac-1267 and immobilized on capron fibers resulted in the formation of a stable microbial consortium and significant decrease of petroleum hydrocarbon, phenol, anionic surfactant, and heavy metal concentrations (Safonova et al. 2004). A pilot plant consisting of UV-irradiation equipment and two successive bioreactors was constructed to treat PCB-contaminated waste oil from high-voltage transformers and condensers, and liquid cultures of *Comamonas testosteroni* TK102 and *R. opacus* TSP 203 were used as inocula for bioreactors providing complete biodegradation of PCBs partially dechlorinated by the UV pretreatment (Kimbara et al. 1998). Groundwater polluted with ethyl *t*-butyl ether (ETBE) and gasoline was treated in the on-site bioreactor inoculated with *R. wratislaviensis*, *R. aetherivorans*, and *Aquincola tertiaricarbonis* cultures, and a 100-fold increase in the abundance of the *ethB* gene encoding a cytochrome P450 involved in ETBE biodegradation was detected by *q*-PCR, thus reflecting the groundwater colonization by the relevant microorganism (*R. aetherivorans*) (Fayolle-Guichard et al. 2012). However, more recently Hatzinger et al. (2017) carried out a pilot propane-fed bioreactor treatment of groundwater contaminated with *N*-nitrosodimethylamine and *N*-nitrodimethylamine left from the use of liquid rocket propellant, and they demonstrated the replacement of initially inoculated propane-oxidizing *R. ruber* ENV425 with native propanotrophs along with the significant increase in microbial diversity and propane monooxygenase abundance over the year of experiment resulted in the efficient contaminant biodegradation.

Biological oxidation of volatile organic carbon vapors by microbial biofilms formed on a solid support in biofilters/bioreactors provides an effective and inexpensive alternative to physicochemical methods (Vinage and von Rohr 2003). A modified rotating biological contactor inoculated with a suspension of *Pseudomonas putida* F1 and *R. erythropolis* PWD1 was proposed by Vinage and von Rohr (2003) for the biological treatment of artificial waste gas polluted with toluene vapors. The proposed system allowed proper control of the biofilm growth and long-term bioremediation performance for a year indicating its feasibility for industrial applications. Borin et al. (2006) investigated microbial succession in a compost-packed biofilter treating benzene-contaminated air and found out that the maximum benzene removal rate strongly correlated with the prevalence of *Rhodococcus* representatives in the bacterial community, thus suggesting their major role in benzene degradation. Aldric and Thonart (2008) evaluated the performance of a water/silicone oil two-phase partitioning bioreactor inoculated with *R. erythropolis* T902.1 cells for

removing volatile organic compounds from gaseous effluents. They reported simultaneous degradation of isopropylbenzene and ethanol by rhodococci, suggesting that ethanol improves contaminant biodegradation process in the bioreactor. Bacterial-fungal consortia containing *Rhodococcus* were successfully tested in column biofilters to treat most common industrial and residential air pollutants, such as volatile organic compounds, PAHs, and hydrogen sulfide (López et al. 2013; Vergara-Fernández et al. 2018). These studies also revealed the ability of rhodococci to survive for a long time in two-component and complex microbial populations of biofilters and to contribute considerably to the contaminant degradation. However, in earlier study of Borràs et al. (2010), a co-culture of *Pseudomonas aeruginosa* CCM 1960 and *R. erythropolis* CCM 2595 inhibited both laccase production and PAH degradation by white-rot fungi in the case of removal of acenaphthylene, benzo[*a*]pyrene, dibenzo[*a*,*h*]anthracene, and benzo[*g*,*h*,*i*]perylene from contaminated soil, thus indicating the need for further research of rhodococcal-fungal interactions during bioremediation.

4 Concluding Remarks

It could be resumed that some success in bioremediation of contaminated environments has been achieved using actinobacteria of the genus *Rhodococcus* either as bioaugmentation agents or members of indigenous microbial communities stimulated by nutrient amendments. Although possible bioremediation applications of genetically modified *Rhodococcus* were not discussed in this review, it should be noted that advanced methods and powerful tools for genetic engineering of rhodococci were developed (see chapter "Diversity and Plasticity of Rhodococcus Genomes" by Cappelletti and Di Gennaro), which could be used in constructing recombinant strains for improved bioremediation inocula. Indeed, the complete nucleotide sequences of numerous *Rhodococcus* genomes provided new insights that could facilitate biotechnological exploitation of this genus. For example, amidase genes from *R. erythropolis* MP50 and *Agrobacterium tumefaciens* D3 co-expressed along with nitrile hydratase from *Bradyrhizobium japonicum* USDA 110 were used for biodegradation of dihalogenated benzonitrile herbicides by recombinant *Escherichia coli* (Pei et al. 2017). Furthermore, *Arabidopsis thaliana* (*Arabidopsis*) was transformed with the hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX)-degrading *xplA* and associated reductase *xplB* from *R. rhodochrous* 11Y in combination with the 2,4,6-trinitrotoluene (TNT)-detoxifying nitroreductase *nfsI* from *Enterobacter cloacae*, resulting in transgenic plants beneficial for remediating RDX- and TNT-contaminated soil and groundwater. However, up today, applications of genetically modified rhodococci in bioremediation fields are limited to laboratory biodegradation studies attempting to reveal their multitudinous catabolic pathways and regulatory mechanisms for different organic contaminants. In the context of future applications of genetically engineered *Rhodococcus* strains in environmental biotechnology, it could be feasible to use biosynthesis products

(e.g., enzymes and biosurfactants) rather than whole rhodococcal cells, which may help to overcome biosafety limitations associated with release of genetically modified microorganisms into open environments. Another possible perspective for molecular genetic approach to be applied to bioremediation is the use of oligonucleotide primers and DNA probes constructed for rhodococcal biodegradation genes to estimate their in situ functional activities (Whyte et al. 2002a; Coffey et al. 2010; Táncsics et al. 2015). These techniques, as well as novel genomic and proteomic methods, could be used to predict bacterial metabolism in contaminated environments and to enhance bioremediation. Moreover, correct prognosis of rhodococcal survival and biodegradation activity in contaminated environments would require further fundamental studies of interactions between *Rhodococcus* cultures introduced and indigenous micro- and macroorganisms using physiological and molecular approaches (Watanabe and Hamamura 2003). Clearly, *Rhodococcus* application in bioremediation of contaminated sites is a promising and evolving field of environmental biotechnology, and its success depends on the increase in our fundamental knowledge of these remarkable bacteria.

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