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Drivers for efficient bioaugmentation and clean-up of contaminated soil

María Balseiro-Romero[1, 2], Lukas Y. Wick[2], Joaquim Vila[3], Magdalena Grifoll[3], José Julio Ortega-Calvo[1]

[1] Instituto de Recursos Naturales y Agrobiología de Sevilla (IRNAS), Consejo Superior de Investigaciones Científicas (CSIC), 41012 Seville, Spain

[2] Department of Environmental Microbiology, Helmholtz Centre for Environmental Research - UFZ, D-04318 Leipzig, Germany

[3] Department of Genetics, Microbiology and Statistics, Section of Microbiology, Virology and Biotechnology, University of Barcelona, 08028 Barcelona, Spain

ABSTRACT

Bioaugmentation constitutes a viable approach for the bioremediation of soils polluted by organic chemicals, but limitations may arise due to the poor *in situ* performance of the inoculated microorganisms. This chapter examines these –poorly understood- drawbacks in the light of the latest advances in microbial ecology and bioremediation strategies. We discuss how biotic and abiotic factors may compromise the establishment and activity of microbial inoculants in soil, as well as how to design efficient inoculants that exhibit increased robustness and dispersal. Innovative approaches could include taking advantage of microbial networks through bacterial consortia with complementary catabolic capabilities, and fungal- and plant-bacterial associations that provide an enhanced bacterial dispersion in water unsaturated soil conditions. We also provide recommendations on the most convenient strategies for inoculant production and application, considering their mass production, the optimal dosing ratios and the optimised use of platforms for microbial action in soil, such as solid carriers (e.g., biochar) and plants.

Keywords: microbial inoculants, bioremediation, ecological stress, fungal highways, microbial dispersal

1. Introduction

Bioremediation exploits the metabolic and enzymatic capabilities of living organisms to transform or remove contaminants from polluted soils (Singh et al., 2017). Generally, bioremediation refers to the use of microorganisms (bacteria and fungi) and often includes microbial interactions with higher organisms such as plants. Typically, bioremediation comprises three different approaches: bioattenuation, biostimulation and bioaugmentation (Alkorta and Garbisu, 2021). Bioattenuation relies on the intrinsic capacity of soil for clean-up and recovery of its functions and diversity in the absence of human intervention. Bioattenuation is hence applied as a cost-effective strategy at sites with relatively low contaminant concentrations that remain confined to avoid pollutant runoff. As soil clean-up is often slow, it is rarely used as the sole treatment approach. In biostimulation the microbiological activity of the indigenous communities is enhanced by changing the environmental conditions to promote pollutant removal. For instance, this includes the addition of nutrients and missing co-factors to balance the soil C/N/P ratios, the correction of pH or the maintenance of favourable moisture or redox conditions.

Bioaugmentation, the subject of this chapter, is based on the addition of microorganisms to soil with relevant metabolic capabilities for the degradation of the target contaminants. This may include the addition of single strains or complex microbial consortia. As a general rule, bioaugmentation is recommended when i) indigenous degrading microorganism are not detected or are found at very low concentrations, ii) a low microbiological activity is observed due to environmental and/or toxicological stresses, iii) a reduction in remediation execution times is required, and iv) one single contaminant is targeted. Inoculants for bioaugmentation include either autochthonous or

allochthonous microorganisms (or their enzymes). In the first case, specialised microbial communities or selected isolates are recovered from the soil to be remediated using enrichment culture techniques. Then, they are amplified in a bioreactor and used to re-inoculate the contaminated soil. In the second scenario, inoculants are based on commercial formulations or microorganisms previously isolated from other contaminated sites to provide the required degradation capacities or other relevant traits, such as biosurfactant production or motility (see section 4). Ideally, microbial inoculants thereby should possess a wide adaptability to environmental conditions (nutrients, aeration, pH, moisture).

An alternative to the traditional cell bioaugmentation approach, genetic bioaugmentation has recently gained some attention. Here, genetically modified microorganisms equipped with mobile genetic elements encoding the enzymes responsible for desired catabolic functions are introduced into soils (Cycoń et al., 2017). These microorganisms act as donors of self-transmissible catabolic plasmids which can subsequently be spread by horizontal gene transfer into well-established, ecologically competitive, indigenous bacterial populations (Garbisu et al., 2017). Despite the high potential of this type of bioaugmentation, there are ethical concerns and legal restrictions regarding the environmental application of genetically modified organisms (Tripathi et al., 2017), and therefore will not be considered here.

The aim of this chapter is to review which are the most positive characteristics to consider when selecting the appropriate inoculants for bioaugmentation strategies in the remediation of soils contaminated with organic pollutants. It further reviews the beneficial aspects of inoculating microbial networks, as well as the inclusion of plants as bioremediation actors. Finally, it reviews the main features to be considered in order to

achieve effective inoculation of contaminated soils and efficient biodegradation of contaminants.

2. Microbial inoculants for bioaugmentation

In recent decades, a large number of contaminant degrading microbial strains have been isolated from soil, their metabolic pathways and genes involved in the degradation identified, and their degradation capacities tested in laboratory systems (e.g. in liquid media, soil microcosms and/or greenhouse mesocosms). Representative recent studies showing the efficacy of bioaugmentation are summarised in Table 1. Also, a number of commercial biological products for use in soil and water remediation are available (Imam et al., 2022).

Isolation of degrading microorganisms from soil is usually achieved by enrichment in mineral medium with the target contaminants as a carbon source, and subsequent plating using a given growth medium. However, culture-based approaches underestimate the actual diversity of natural communities and often neglect potential interactions between their components (Vila et al., 2015). Standard culture conditions and protocols preferentially select fast-growing microbes that typically grow at high nutrient availability, mesophilic temperatures or neutral pH (Kaminsky et al., 2019). Aware of these limitations, microbiologists have developed new cultivation approaches to increase the biodiversity of cultivable microbes such as molecular community analysis - directed isolation. In this approach key microbial components of enriched microbial consortia are identified based on their relative abundance or by metagenomic analysis and isolation media are designed to recover them as pure cultures by providing selective conditions (Vanbroekhoven et al., 2004) or required nutritional factors (Stevenson et al., 2004). A common limiting growth factor in the widespread auxotrophy for vitamin B12 detected

in soil degrading bacteria (Jimenez-Volerink et al., 2023a). More sophisticated approaches take into account microbial interactions for efficient biodegradation (Espinosa-Ortiz et al., 2012). For example, in 2012 Furuno et al. proposed the use of mycelial-bound bacterial dispersal as a selection criterion for the targeted isolation of specialised bacteria able to access low availability substrates. Based on this idea, in situ systems for the isolation of degrading consortia have been developed (Junier et al., 2021). Other approaches are based on single cell manipulation (Huys and Raes, 2018), including free cell sorting (flow cytometry or Raman-activated cell sorting) (Shan et al., 2023) or growth of trapped cells in miniaturised microenvironments (microfluidic devices or gel microdroplets) (Berdy et al., 2017).

An ideal microbial inoculant should produce sufficient biomass for large-scale field applications, so that growth can be easily scaled up to large-volume bioreactors, while maintaining an appropriate balance between the economic feasibility of production and the specific growth conditions of the promising inoculant. Therefore, the selection of inoculants should take into account their positive metabolic and behavioural traits, their performance and viability under field conditions, and the economic feasibility of biomass production. In the next sections we review first the limitations that microbial inocula face when introduced into soils (section 3) and the desirable traits to overcome those limitations (section 4).

3. Factors conditioning the performance of microbial inoculants in soil

Most bioaugmentation studies demonstrating the efficacy of bioaugmentation have been carried out at microcosmos or mesocosm scale, often involving modification of the original soil conditions (i.e. soil sterilisation, spiking target contaminants). Table 1 exemplifies some of these cases. Only limited examples are available on the efficacy of soil bioaugmentation in real field applications as reviewed by O'Callaghan et al. (2022).

128 The performance and survival of microbial inoculants in soils is influenced by many
129 biotic and abiotic factors (Ghosh et al., 2021) (Figure 1). Despite the apparent simplicity,
130 bioaugmentation at field sites is subject to several limitations including (i) poor
131 establishments and survival of inoculated degraders in soil in response to ecological stress
132 or microbial competition (Gao et al., 2022), (ii) the inactivation of externally added
133 enzymes due to site specific conditions (Saravanan et al., 2021), and (iii) the limited
134 dispersal of inoculants towards the contaminant source within the soil matrix (Zhong et
135 al., 2017). Therefore, the performance of highly efficient degrading strains under
136 laboratory conditions may be poor or highly variable in soils of different physicochemical
137 properties or when exposed to varying environmental conditions. For example, a recent
138 study compared the total petroleum hydrocarbon (TPH) remediation effectiveness of a
139 soil in presence and absence of bioaugmentation with an autochthonous degrading
140 microbial consortium; the data showed that despite effective colonisation of the soil by
141 the inoculants, TPH removal was lower in the bioaugmented soil than in the soil
142 biostimulated with a nutrient solution only. These results were attributed to a dramatic
143 reduction in soil biodiversity after bioaugmentation, resulting in a predominance of
144 *Pseudomonas* that did not perform well at the given soil conditions (Wu et al., 2019).

145

146 ***3.1. Biotic factors***

147 Microbial populations within soil microbiome are in continuous interaction under
148 synergistic or antagonistic relationships that are the major driver for community assembly
149 and stability (Bahram et al., 2018). Key antagonistic interactions that affect the
150 performance of microbial inoculants include the (i) competition with autochthonous
151 microbes for carbon and other limiting nutrient sources, (ii) predation by protists or
152 bacteriophages, or (iii) inhibition by antimicrobial compounds (Albright et al., 2021). The

153 successful colonisation and establishment of an inoculum will therefore be influenced by
154 the biodiversity of microbial species in the soil and the availability of nutrient resources.
155 Higher diversity is generally associated with higher soil functionality (Delgado-
156 Baquerizo et al., 2016) and better use of nutrients and carbon sources, which will reduce
157 the unoccupied niches to be filled by allochthonous inoculants. In addition, when
158 inoculants are closely related to indigenous species, there is a greater likelihood of niche
159 overlap and increased competition, compromising the successful establishment of
160 inoculated populations (O'Callaghan et al., 2022). Niche adaptation of microbial
161 inoculants is a very important issue to consider when selecting an inoculant (Vogel,
162 1996). To minimise the effects of niche competition and decrease potential negative
163 impact on the indigenous microbiota and macrobiota, Sprocati et al. (2012) suggested the
164 use of inoculants phylogenetically related to the main taxonomic groups that constituted
165 the autochthonous microbial community structure of the soil to be remediated.

166 Following inoculant application, cell numbers will certainly be at a disadvantage
167 compared to autochthonous communities. In addition to cell death following application
168 stress, microbes are likely to suffer from biomass loss due to cell predation
169 (bacteriophages, protozoa). Therefore, the design of appropriate inoculation strategies
170 (discussed in section 6) will be key to improve the efficacy and competitiveness of the
171 inoculants applied (e.g. inoculation close to roots in rhizo-remediation strategies,
172 successive re-inoculations, encapsulated inoculation, etc.).

173

174 ***3.2. Abiotic factors***

175 Soil properties, like soil porosity and composition, temperature, pH level, redox
176 potential, or moisture content, and the contaminant's concentration and toxicity are the
177 main abiotic factors that affect inoculant performance. To predict the success of

bioaugmentation in soil, Horemans et al. (2017) developed a soil-bacterium compatibility model that considers these factors.

A major factor influencing contaminant degradation, and hence the potential success of bioaugmentation, is bioavailability. Bioavailability is defined as the amount of a chemical available for biotransformation or toxicity. In terms of biodegradation, bioavailability refers to the fraction of a chemical that can be taken up or transformed by the active organisms during the course of bioremediation. Both physical availability and microbial activity are microbial system properties that drive pollutant bioavailability (Wick, 2020). Hydrophobic chemicals, for instance, may interact so strongly with soil matrices that they may become poorly available or not available at all for microbial attack. Next to the physicochemical properties of a contaminant, other factors related to the characteristics of the soil or the microorganisms may influence the availability and transfer of a chemical to degrading organisms (Niqui-Arroyo et al., 2011). The composition of the soil (e.g., mineral and organic matter content), its structure (e.g., porosity) or hydrogeology (e.g., contaminant mixing) can influence the dissolution, desorption, diffusion and distribution of a contaminant. The degree of interaction of the organic contaminants with the soil matrix will also be influenced by the time of residence due to a phenomenon known as contaminant ageing, a concept that has stood well since its introduction in the early 2000s (Alexander, 2000). Increasing time of contact of the contaminant with the soil will result in a more irreversible sorption and a higher penetration into the finer soil aggregates, thus decreasing the accessibility of bacteria to the contaminants.

During soil bioremediation, a suitable strategy to enhance the bioavailability of the slowly desorbing organic contaminants is the application of synthetic surfactants, which has resulted in variable outcomes (Tiwari & Tripathy, 2023). Surfactants may be directly

toxic to the soil microbiota due to their interactions with cell membrane integrity, but may also cause an indirect toxic effect by increasing the bioavailable concentrations of the pollutants or their transformation products. The synthetic surfactants most frequently employed in bioremediation scenarios belong to the non-ionic group, which generally show less toxic effects to the degrading microbial communities than cationic and anionic surfactants. The lower critical micelle concentration of this group facilitates their solubilisation efficiency at lower concentration doses, which reduces the environmental impact of surfactant application and the demands of nutrients and electron acceptors (e.g., oxygen) caused by the biodegradation of the surfactant. A few studies indicate that nonionic surfactants may be more efficient at a second stage of bioremediation, when the most bioavailable pollutants have been removed. For example, the nonionic alkyl poly(ethylene glycol) ether surfactant Brij 35 inhibited the biodegradation of high-molecular-weight PAHs (HMW-PAHs) in an untreated creosote-polluted soil, but enhanced biodegradation of HMW-PAHs in a manufactured gas plant (MGP) soil that had been extensively bioremediated in biopiles during several years, leading to a slow-desorption profile (Bueno-Montes et al., 2011). Similarly, another non-ionic surfactant from the same family, Brij 30, enhanced the desorption and biodegradation of residual PAHs in a MGP soil previously treated in an aerobic reactor, whereas no effect was observed with the untreated soil (Zhu and Aitken, 2010). A promising alternative to chemical surfactants in bioremediation is the use of biologically produced surfactants. Among these, rhamnolipid, an anionic glycolipid biosurfactant produced by the bacterium *Pseudomonas aeruginosa*, is perhaps the most studied biosurfactant. It has already been shown to enhance the desorption and biodegradation of aged pyrene (Congiu and Ortega-Calvo, 2014), and to efficiently reduce end-point PAH concentrations when applied to soils after conventional bioremediation (Posada-Baquero et al., 2019a, 2019b) and

phytoremediation (Posada-Baquero et al., 2020). After the extensive literature review of bioaugmentation cases performed in this chapter, we did not find reported studies that combined this strategy with surfactant application. In accordance to the studies above, it is conceivable that surfactants can increase biodegradation rates once the inoculants have been established in the soil, minimising possible negative impacts due to surfactant toxicity, and allowing for their actions on poorly available, residual pollutants. Alternatively, soil bacteria have developed mechanisms to overcome the limitations posed by low substrate bioavailability, including the production of surface-active compounds to increase *in-situ* the contaminant mass transfer into the water phase by pseudo-solubilization; their active movement toward the source of contaminant favoured by chemotactic behaviour and/or their migration along fungal mycelia; or the increase of organic compound bioavailability by the direct contact of bacterial cells with the non-aqueous phase liquid (NAPL) of oil spill. Bioaugmentation with biosurfactant producing or chemotactic motile bacteria can help to tackle poorly available fractions of contaminants (Ortega-Calvo et al., 2020).

Contaminant concentration is another aspect to consider for successful soil inoculation. When pollutant concentrations exceed tolerance ranges for inoculants, their toxicity may lead to low survival rates, and poor performance of the inoculants. In those cases, soil pretreatment may be necessary, including physical and chemical-based remediation techniques, such as free-phase removal by soil flushing or surfactant slurping, chemical oxidation, soil vapour extraction, electrokinetic separation or soil fracturing. Conversely, low concentrations of organic contaminants can lead to extremely slow biodegradation rates due to the lack of microbial activation and enzymatic induction. This is of major relevance for chemicals of particular concern from a toxicological point of view, such as the well-known carcinogen benzo(a)pyrene. This highly hydrophobic

PAH can be found in hydrocarbon contaminated soils at bioavailable concentrations below the threshold values necessary to induce the expression of specific degradative genes, but at total concentrations well above the regulated reference levels. In such cases, inoculation with microorganisms able to cometabolize these minority pollutants while degrading other contaminants present at higher concentrations will be beneficial.

The presence of transformation products, eventually more toxic than the parent molecules, could also have inhibitory effects. That is the case of polychlorinated biphenyls (PCBs), whose hydroxylated intermediate metabolites are more toxic than the parent molecule (Passatore et al., 2014). Subramanian et al., (2017) observed that while parent PCBs and higher-chlorinated hydroxylated derivatives were not toxic for *Arabidopsis thaliana*, lower-chlorinated hydroxylated derivatives significantly inhibited the germination rate and plant growth.

Complex contaminant mixtures, such as the co-existence of heavy metals and organic contaminants (which is the common situation of many brownfields and degraded agricultural soils worldwide), may also affect the microbial activity of inoculants presenting additive toxicological effects (Thavamani et al., 2011). Inhibition of microbial activity by metals has been associated with the decrease of enzyme activity and the oxidative pressure on microorganisms (Zhang et al., 2020). Metals can also influence the sorption/desorption kinetics of soil contaminants by the formation of organometallic complexes that modify the solubility, bioavailability, and toxicity of original pollutants (Ye et al., 2017).

The physico-chemical properties of the soil strongly influence inoculant performance. Efficient bioremediation of organic contaminated environments has been observed in deserts and polar areas, however, temperature will affect the growth, survival, metabolic rates and enzymatic activity of the commonly mesophilic commercial microbial

inoculants. Moreover, temperature could affect the physical properties of contaminants, such as viscosity, volatility, water solubility and bioavailability. Extreme temperature regimes are indeed a challenge for bioaugmentation, with allochthonous microbial inoculants being easily outcompeted by native extremophilic microbial communities presenting specific adaptive features to extreme conditions. In such cases is definitively recommended the use of autochthonous microbial inocula.

In addition to its obvious effects on cell metabolism, pH may have an influence on the solubility of nutrients and trace elements, hence having a potential effect on their availability for microbial uptake and/or their toxicity. Soils from industrial sites polluted by organic contaminants may contain demolition materials, such as concrete and bricks, which can increase the soil pH as a result of leaching. On the other hand, at coal mine sites, extremely acidic conditions can be encountered as a result of acidic drainage from the oxidation of sulphites in coal spoil heaps. To implement bioaugmentation in those sites, it is common to adjust the pH of the site using chemicals (e.g., ammonium sulphate or ammonium nitrate for basic soils, and lime for acidic soils).

Activity of microbial inoculants will be highly dependent on soil water content. In highly saturated soils the rate of organic matter decomposition is decreased due to low oxygen supply. Conversely, a low water content decreases soil microbial activity by reducing diffusion of soluble substrates, microbial mobility and intracellular water potential (Alkorta et al., 2017). Therefore, during bioaugmentation water content should be controlled, being typically adjusted between 20-60% of the soil water holding capacity.

The concentration of oxygen and the redox potential will determine the performance of aerobic or anaerobic inoculants. During the aerobic degradation of organic contaminants, oxygen is always supplied either by physical or by chemical methods. In aerobic degradation, soil organic pollutants generally serve as a carbon source for

303 degrading microbial inoculants. Assuming an elemental cell composition of
304 $C_{60}H_{87}O_{23}N_{12}P$, nitrogen and phosphorus are especially limiting factors for the complete
305 assimilation of organic contaminants. Other micronutrients, such as potassium, iron,
306 sulphur and chromium, are necessary cofactors for some enzyme activities, and therefore
307 they can limit microbial-driven degradation in poor soils. Microbial inoculants are usually
308 included in formulations that also contain potentially limiting nutrients and cofactors, or
309 are added in combination with them. However, nutrient addition should be conducted
310 with caution, as the optimal C:N:P ratios (Leys et al., 2005) are variable for each site and
311 high nutrient levels may negatively impact the biodegradation of organic contaminants
312 (Ghosal et al., 2016). The type of appropriate nutrient formulation (i.e. hydrophilic,
313 oleophilic or slow-release) may also be considered (Coulon et al., 2012). Therefore, the
314 concentration and type of nutrient formulation should be tested in a case-specific manner
315 considering the C availability and original nutrient status of the site.

316

317 **4. Desired traits for more efficient microbial inoculants**

318 The design of "tailored inoculants" using previously studied microbial strains is of
319 great importance in order to guarantee their performance in soil. Logically, the major trait
320 to consider when selecting an inoculant is the degradation efficiency of the target
321 contaminant. However, there are other positive traits that will be beneficial when
322 microorganisms are inoculated in soil, mainly related to the accessibility and availability
323 of the pollutant, and the ability to survive under a range of environmental conditions and
324 pollutant concentrations (Cycoń et al., 2017).

325

326

4.1 Robustness

To achieve an effective bioaugmentation, microbial inoculants should have the ability to establish, proliferate and be active after *in situ* applications. Sourcing degrading microbial strains for bioaugmentation is typically achieved by selective enrichment. Strains from a polluted sample, either from the site to treat or from another historically polluted site, are enriched to grow in minimal culture media using the target contaminant as the sole carbon source. This procedure results in the selection of strains that express the required degradation ability under the very specific conditions of the enrichment culture, generally leading to the isolation of fast growing mesophilic copiotrophs (Kaminsky et al., 2019). Other traits that are also required for strains to be competitive under the fluctuating environmental conditions (e.g. moisture, nutrients, redox, pH and osmotic factors) or the competition from indigenous microbial populations and potential predators are seldom considered (Thompson et al., 2005). Thus, microbial inoculants frequently fail to establish or confer long-lasting modifications to soil microbiome and ecosystem function (Albright et al., 2021). Several strategies have been proposed to improve inoculant establishment, such as i) increasing dosage, frequency or mode of delivery of the inoculant; ii) providing increased resistance to environmental disturbances by creating a protected physical space for inoculant implantation, using an inoculant pre-adapted to the target environment, or adding inoculant-adapted specific resources (e.g. prebiotics or growth factors); or iii) conferring inoculant increased resistance to biotic interactions by selecting microorganisms tolerant to antagonistic interactions such as competition, predation or antimicrobial production, by providing a protected environment for their development, or by co-inoculation with other species that provide ecosystem services necessary for their appropriate development (Albright et al., 2021). Among these strategies, there is increasing evidence describing the benefits of biomass immobilisation

using different supporting carriers that act as a protective environment in front of extreme environmental conditions, also preventing the loss of free cells during bioaugmentation. For example, Laothamteep et al. (2022) successfully applied a zeolite-immobilised bacterial consortium during the bioaugmentation of a crude oil contaminated soil, and Zhai and colleagues (2023) used biochar as carrier for an herbicide-degrading bacterium during the bioaugmentation of a nicosulfuron-contaminated soil.

A suitable strategy to improve inoculant implantation is the use of autochthonous microbial populations selected on the basis of both their catabolic and ecological traits. Relevant contaminant-degrading soil bacteria with competitive traits in a particular environment could be directly identified *in situ* by means of molecular methods. Stable isotope probing (SIP), relying on the spiking of ^{13}C-labelled tracers and further analysis of ^{13}C-enriched biomolecules (DNA, RNA, proteins or PLFA) is ideal for identifying microbes effectively assimilating contaminant carbon under the pressure of site-specific environmental stressors (Uhlik et al., 2013). For instance, a recent work applying SIP combined with metagenomics identified that the major active degraders of linuron in a spiked agricultural soil differed from those selected after an enrichment process in liquid culture (Lerner et al., 2020). Information gathered from SIP-metagenomic analysis could be further integrated for the molecular directed-isolation of identified degraders (Vila et al., 2015). Genome-wide metagenomics, can provide the theoretical understanding of the cultivation requirements of major identified uncultured degraders from the functional interpretation and metabolic reconstruction of metagenome assembled-genomes (Liu et al., 2022). This information should provide greater possibilities to capture uncultured and often elusive bacteria, thus providing inoculants with optimal degrading and ecological traits for effective soil colonisation. The usefulness of the SIP-based identification strategy was recently demonstrated by Luo et al. (2021). Using DNA-SIP, they first

identified a member of *Ralstonia* by DNA-SIP as the major phenanthrene and biphenyl degrader in contaminated industrial waste-waters. After its isolation, they inoculated with this autochthonous population to enhance the biodegradation of PAHs during the remediation of the contaminated waste-water.

4.2. Motility and dispersal

The high degradation capacity of an inoculant will be worthless if there is no close contact between the microbial cells and the pollutant in the soil. It is generally assumed that after inoculation bacteria will reach and process the contaminant, but this might not always be true, leading to a poor remediation efficiency, despite the use of powerful degraders and favourable biostimulation conditions.

Pollutants are subjected to a high variety of macro and microscale transport processes, mainly related to the movement of water within soil pores, the contaminant adsorption to soil particles and soil organic matter, their entrapment into NAPLs or soil aggregates, or the limited rates of transfer to the water phase. Within this movement, pollutants may casually reach microbial hotspots and be degraded there. In a similar way, part of the inoculated microbes can migrate with water flows and reach pollutant hotspots. These carbon and cell dispersion processes are well-studied at a macroscale dimension (Vogel et al., 2015). Processes involved at the microbial scale are “hidden” and still less well-known (Baveye et al., 2018), however, they can be very significant for enhancing pollutant biodegradation and should be carefully considered.

Microbes are passively dispersed within soil as particles either by Brownian diffusion, by water movement or by surface motility mechanisms such as sliding (Hölscher and Kovács, 2017) or gliding (Tchoufag et al., 2019), powered, respectively, by the pushing

401 forces of dividing cells and of the excretion of microbial extracellular polymeric
402 substances. However, those mechanisms are not significant for long distance dispersion
403 compared to active, flagellar motility. Flagellated microbes can develop chemotactic
404 responses which allow them “moving” (by swimming or swarming) and access
405 contaminants in the drive of a chemical up-gradient (Krell et al., 2013; Ren et al., 2018).
406 It is known that bacteria have very high cell adsorption and deposition rates in soil when
407 they are moving together with water flows, but it has been demonstrated that chemotaxis
408 and active dispersal can reduce this deposition. Random cell swimming, which is
409 typically characterised by short paths and spontaneous changes in the directions of
410 swimming, tends to be smoothed in the presence of chemoeffectors and favours long-
411 distance bacterial dispersion within porous environments (Ford and Harvey, 2007;
412 Jiménez-Sánchez et al., 2012), facilitating the access to distant pollutant sources and thus
413 enhancing biodegradation rates (Jimenez-Sanchez et al., 2018; Rolando et al., 2020;
414 Castilla-Alcantara et al., 2023b). It has been demonstrated that non-motile microbes can
415 be co-transported together with motile bacteria, and some mechanisms have been
416 identified, including mechanical pushing, direct surface attachment to motile cells, direct
417 attachment to flagella, and cell internal transport (Hagai et al., 2014; Muok and Briegel,
418 2021). A recent study confirmed that the motile and chemotactic bacteria *Pseudomonas*
419 *putida* G7, was able to co-mobilize non-motile bacteria (*Mycobacterium gilvum* VM552,
420 a polycyclic aromatic hydrocarbon-degrader, and *Sphingobium* sp. D4, a
421 hexachlorocyclohexane-degrader) through micrometre sized pores, only when they
422 sensed a carbon gradient through the pores (Balseiro-Romero et al., 2022).

423 The processes previously described are limited to soil saturated pores, but unsaturated
424 conditions will also be present in soil environments. Obviously, bacterial cells will not be
425 able to swim and disperse into pores filled with air. However, fungal mycelia (structures

of high fractal dimension and high external surface areas) are able to cross air–water interfaces, interconnect air-filled soil pores, and colonise small soil micropores (hyphae with diameters varies from 2 to 10 μm). The formation of these networks allows fungi to better exploit soil resources, including soil pollutants, and enables the internal long-distance transport of chemicals, which gives them access to low bioavailable fractions in remote soil pores (Harms et al., 2011; Fester et al., 2014).

Some studies have shown that fungal mycelia can also act as bacterial dispersal networks ("fungal highways") (Kohlmeier et al., 2005).I In addition to the improvement of pollutant transport through linked cytoplasmic channels, mycelial networks provide thin aqueous films which may induce chemotactic responses towards pollutant hotspots of otherwise immobilised bacteria (Banitz et al., 2011; Furuno et al., 2012; Schamfuß et al., 2013; Sungthong et al., 2017). Although the tortuous pathways in porous media reduces effective bacterial movement, it seems sufficient to bridge distances relevant for accessing contaminants (Harms & Wick, 2006). Mycelia thus promote the dispersal of active bacteria towards soil contaminants and, hence, efficient bioremediation (Wick et al., 2010). Next to enabling the efficient colonisation of subsurface interfaces and new habitats, mycelia also promote the transport of water, nutrients and contaminants by their cytoplasmic streaming and, by partially sharing them with bacteria in the mycosphere (i.e. the habitat surrounding and formed by mycelia), increase the activity and functional stability of dispersing bacteria. The mycosphere hence is a unique and highly dynamic bacterial habitat and a hotspot for contaminant biotransformation (Wick, 2020; Khan et al., 2023).

4.3. Biosurfactant production

The secretion of surface-active compounds and/or bioemulsifiers may increase the bioavailability of the contaminants by enhancing their apparent solubility (through

451 micellar solvation), and/or by dispersing the soil matrix or the non-aqueous liquid phase
452 (through the modification of interfacial tensions) (Bezza and Nkhalambayausi Chirwa,
453 2016; Lamichhane et al., 2017; Pacwa-Płociniczak et al., 2011; Singh et al., 2017). The
454 secretion of extracellular polymeric substances involved in the development of biofilms
455 also facilitates microbial attachment to contaminants, helping to overcome mass-transfer
456 restrictions in low bioavailability matrices (Zhang et al., 2015). However, this can
457 negatively affect dispersion, since deposition rates will be favoured. The establishment
458 of biofilms also favours the co-adhesion of mixed bacterial communities (favouring
459 synergic metabolism) and gene-transfer to enhance degradation (Ortega-Calvo et al.,
460 2013).

461

462 **4.4 Production of extracellular enzymes**

463 The secretion of diffusible extracellular enzymes is also a positive trait to enhance the
464 access to remote and non-accessible contaminants. This may be referred to as "enzyme-
465 assisted bioremediation". Most extracellular enzymes can diffuse away from the cell
466 producing them (Burns et al., 2013), reach the pollutants, and enhance their availability,
467 since the cell uptake of the pollutant is not necessarily required (Van Hamme, 2004).
468 Some of these enzymes can be more resilient than the intracellular equals and have
469 structural modifications that allow them to better support adverse environmental
470 conditions, such as temperature or pH variations (Burns et al., 2013). Allison and co-
471 workers (Allison et al., 2011), also hypothesised that enzyme diffusivity can increase as
472 substrate availability decreases, enhancing the availability of distant pollutant sources.

473

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5. Co-inoculants: the benefits of microbial networks

Inoculant formulations used in bioaugmentation strategies can consist of a single strain (bacteria, yeasts or fungi), a synthetic consortium (obtained by combination of complementary isolated strains), or a non-defined microbial consortium developed by enrichment procedures (Xu and Zhou, 2016). There is a general agreement that the use of microbial consortia as soil inoculants can provide functional benefits over single-strain inoculants. Due to the complexity of soils, the variety of pollutants and the diversity of niches to be colonised, the inoculation of microbial consortia will increase the catabolic potential and the success of inoculant survival and establishment in soils. These beneficial effects may originate from different factors, such as synergistic catabolic functionality, cross-feeding relationships between members, improved contaminant bioavailability by biosurfactant solubilization, or facilitated microbial dispersion by co-transport with chemotactic bacteria or through “fungal highways”.

The use of combined inoculants including bacterial, fungi and/or yeasts, appears as an attractive strategy in front of complex contaminant mixtures. Complex pollutants impose stress conditions on a single species, hindering their performance. In contrast, microbial consortia tend to show resistance and multi-functionality as varied species work together to efficiently utilise all forms of substrates. The use of microbial consortia with different members possessing complementary catabolic capabilities may be preferred to treat soils contaminated with complex organic mixtures. For example, crude oils and derivatives encompass a great diversity of chemical classes, including aliphatic and aromatic compounds. Microbial members within enriched oil-degrading microbial consortia are specialised in the degradation of different constituents within the mixture (Vila et al., 2010; Tauler et al., 2016). The co-inoculation of a variety of degraders with overlapping catabolic capabilities may also be beneficial in order to achieve a faster and

more efficient degradation of organic mixtures. Functional redundancy has been associated with community resilience and adaptability to varying environments (Louca et al., 2018), and seems to be a common trait in microbial communities from contaminated soils (Guazzaroni et al., 2013).

When facing a complex organic mixture, a single bacterial strain may act on a variety of components without being able to completely mineralize them. The attack could involve a cometabolic oxidation or a partial degradation of the contaminant molecule, leading in both cases to the accumulation of polar transformation products. Several authors have observed the formation of mutagenic and/or (geno)toxic metabolites during bacterial degradation. Chibwe and co-workers (Chibwe et al., 2015) observed that the increased toxicity of a PAH contaminated soil after aerobic bioremediation could be associated with the accumulation of hydroxylated and carboxylated metabolites of PAHs. Further research from Tian and co-workers (Tian et al., 2017) identified a metabolite derived from the bacterial dioxygenation of the PAH pyrene as a major contributor to the increased genotoxicity of bioremediated soil. Many other examples of metabolites more toxic than the parent molecule can be found in the literature, such as the transformation products of trinitrotoluene (Neuwoehner et al., 2009) and several pesticides (Du et al., 2015; Enhui et al., 2014; Giacomazzi and Cochet, 2004). In those cases, synergistic interactions may take place within microbial communities, where some populations can specialise in the further utilisation of the transformation products (Jiménez-Volkerink et al., 2023a).

Cross feeding relationships and nutritional interdependencies frequently occur within soil microbial communities, mainly associated with the exchange of vitamins, amino acids and other cofactors (Zengler & Zaramela, 2018). For example, vitamin B12 is an essential cofactor involved in the synthesis of nucleotides and amino acids, but only a

relatively small subset of soil bacteria is capable of synthesising it (Lu et al., 2019). Auxotrophy for some aminoacids such as methionine, playing an important role in the initiation of translation and as constituent of proteins, have also been documented (Perruchon et al., 2020). Recent metagenomic characterization of a soil microbial consortium specialised in the degradation of PAH-transformation products has revealed the complex nutritional interdependencies between the members of the community (Jiménez-Volkerink et al., 2023b). The capability to synthesise vitamin B12 and methionine was limited to a restricted number of components within the community that were unable to degrade the contaminant. Therefore, a strategy to consider in order to improve the establishment of microbial inoculants can be the design of synergic co-inoculants, involving highly efficient contaminant degrading communities and facilitative accompanying populations to fulfil their nutritional requirements.

Tailored co-inoculants could also be designed to improve the dispersal of contaminant-degrading communities in the soil, and to increase contaminant bioavailability. For example, a good biosurfactant producer, even without specific degrading capabilities may be beneficial as inoculant. The biosurfactant produced may lead to a higher release of the contaminants from the soil sorption sites, and therefore, enhance the bioavailability for the degrading strains. This strategy was successfully applied by Alvarez et al. (2022) during the bioaugmentation of a HCH-contaminated soil. The co-inoculation of the HCH-degrading strain *Sphingobium* sp. strain D4 with two biosurfactant producing strains, resulted in enhanced mobilization and therefore degradation of HCH isomers in the soil. On the other hand, the inoculation of degraders together with motile and chemotactic bacteria, and/or fungi may be beneficial to improve bacterial dispersion. The effectiveness of motile and chemotactic *Pseudomonas putida* G7 to co-mobilize PAH and lindane degraders in porous media has been shown recently

(Balseiro-Romero et al., 2022). The fungal networks have also been studied and it has been demonstrated that they can enhance the dispersion of bacteria, especially important under unsaturated conditions (Kohlmeier et al., 2005; Simon et al., 2015; Warmink et al., 2011). Fungal-bacterial co-cultures hence are particularly thought to be efficient for environmental remediation as mycelia may act as dispersal vectors for pollutant degrading bacteria in unsaturated porous media (Harms et al., 2011; Espinosa-Ortiz et al., 2022). Many fungi also possess extracellular enzymes (e.g. lignin peroxidases, laccases) that non-specifically, and often co-metabolically, degrade complex pollutant molecules (Magan et al., 2022) even at low concentrations by utilising other available carbon sources such as plant material or bacterial exudates. Fungal metabolites thereby can serve for bacterial growth and co-metabolic contaminant degradation (Khan et al., 2023). Liu and co-workers (Liu et al., 2017) reached a 57.72% of TPH degradation after 30 days during the bioremediation of a soil inoculated with *Pleurotus ostreatus* P1 and *Bacillus licheniformis* Y-1. The fungal enzymes were able to partially decompose petroleum hydrocarbons in soil, and generated simpler carbon chains which were more easily utilised by the bacterial cells. Jiang al., (2015) developed a remediation procedure of a Cd-PAH co-contaminated soil using the combined remediation effect of *Pleurotus cornucopiae* and *Bacillus thuringiensis* FQ1. They found that besides the PAH removal efficiency, the bacterial inoculant enhanced the fungal growth and Cd accumulation, and alleviated the oxidative stress induced by the contaminants. The relative contributions of the fungal-bacterial partners to degradation and soil detoxification processes have been, however, still poorly quantified. Likewise, knowledge on the spatiotemporal functional stability of fungi and fungal-bacterial associations is limited (Wick, 2020). As bacteria and fungi may also express antagonistic relationships, studies on the compatibility, performance and stability of fungal bacterial co-inocula are needed. In addition to the

antibacterial activity of fungal metabolites, bacteria may also express antifungal activity by producing extracellular lytic enzymes, siderophores, salicylic acid, antibiotics, or volatile metabolites, such as hydrogen cyanide. The use of 'targeted isolation' strategies for specialised fungal-bacterial consortia (see above; Furuno et al., 2012; Simon et al., 2015; Junier et al., 2021) is hence highly recommended.

6. Strategies for inoculant production and application

Promising isolates or consortia should be selected as bioaugmentation inoculants for a particular site based on the required metabolic and behavioural capacities, as well as their expected survival and adaptability to the conditions in the field. Prior to their field application, selected inoculants have to be produced in large batch cultures. In industrial-scale fermenters at least 10–20 generations of growth from seeded stock isolates are required to achieve marketable biomass (Takors, 2012), so an ideal soil microbial inoculant should grow fast to minimise production costs and risk of contamination, and be genetically stable under culture conditions. High genetic plasticity, high mutation rates and low plasmid retention could decrease the downstream inoculant functions in soil. However, traits that benefit batch production may prove detrimental during inoculant establishment and function in the soil matrix. Liquid culture growth favours planktonic phenotypes, potentially impeding surface attachment and colonisation in terrestrial systems.

Following mass production, an ideal soil microbial inoculant should remain viable during long term storage and transport to the site. Microbes compatible with dry formulations are often preferred, as liquid formulations typically have shorter half-lives, higher contamination risk, and require refrigeration. Stress-tolerant spores produced by

some bacteria (e.g., *Bacillus*) and fungi are particularly amenable to dry formulation and promote inoculant persistence in the environment.

The dosing ratio and scheme, and the mode of inoculation are relevant factors that can influence bioaugmentation performance. The amount of inoculum should be sufficient to guarantee the coverage of the whole contaminated areas, and to favour an adequate bacterial dispersion on soil pores. It is generally accepted that inoculum dosing should target a minimum inoculant concentration of 10^6-10^7 cells/g of soil in the whole treatment area, that should be maintained throughout the treatment. Recent literature reviews on successful soil bioaugmentation experiences refer inoculum concentration values ranging between 10^7 to 10^9 cells/g for oil-contaminated soils (Gupta et al., 2022), and between 10^6 to 10^{10} cells/g for pesticide contaminated agricultural soils (Cycoń et al., 2017). In general, increased microbial dosing should improve bioaugmentation performance, however, an appropriate compromise should be achieved between remediation effectiveness and costs.

The mode of inoculation will significantly influence the distribution of microbial inoculants in the contaminated site, which is of major importance for the adequate contact between the microbes and the pollutants. Inoculants are generally applied as a liquid suspension, which can be homogeneously distributed on top of the soil or injected at different depths. Inoculation strategies should also be designed in order to overcome the above mentioned environmental pressures (see section 3) and improve the survival and establishment of the inoculant. Some authors have highlighted the positive role of adding the inoculants immobilised on solid carriers prepared under optimal laboratory conditions (Schoebitz et al., 2013). Those solid carriers act as a protection for the cells during the first stages of the bioremediation procedures, improving their adaptation to soil conditions (Wang et al., 2023), and increasing their survival rate. Different immobilising materials

have been studied, including biochar (Deng et al., 2021), polyvinyl alcohol (Chen et al., 2021), zeolite (Laothamteep et al., 2022), sodium alginate (Dou et al., 2021) or graphene oxide (Ren et al., 2022), among others. The contaminated soil itself may also act as a carrier for the inoculants. By using this carrier, the microbes can be pre-adapted to the pollutant as well as other soil properties (Innemanová et al., 2018).

7. The role of plants in bioaugmentation efficiency

When plants are included as actors in bioremediation (the so-called, plant-assisted bioremediation), they have many positive influences in the nearby environment which may enhance the performance of microbial biodegradation. Plants can enhance microbial biomass production by providing readily available carbon sources, nutrients, oxygen and favourable redox conditions (Wang et al., 2011). Furthermore, they can stimulate pollutant degradation due to the secretion of a variety of organic substances, such as root exudates and secondary plant metabolites. Those substances with a similar chemical structure to the pollutants, may act as co-substrates and stimulate microbial pollutant degradation (Jha et al., 2015; Wojtera-Kwiczor et al., 2014). Numerous studies have also demonstrated the effect of root exudates, which are mainly composed of low-molecular weight organic acids (as well as proteins, mucilage, sugars, amino acids, or phenolic compounds), in enhancing the desorption of organic contaminants in soils (Álvarez et al., 2012; Balseiro-Romero et al., 2014; Gao et al., 2017; Martin et al., 2014; Mitton et al., 2012). Desorption enhancement may in turn improve pollutant bioavailability to microorganisms and, therefore, degradation efficiencies (LeFevre et al., 2013). Solubilization of residual fractions of hydrophobic pollutants (PAHs) can be increased by microbial biosurfactants applied to soils with a slow-desorption profile, in a process favoured by the presence of decaying plant biomass (Posada-Baquero et al., 2020). In

addition, plants may benefit from their associated-bacteria possessing degradation potential, leading to enhanced mineralization and lowering both the phytotoxicity and the evapotranspiration of volatile pollutants.

All of the traits discussed above can facilitate the integration of plants in the bioaugmentation scenarios considered in this chapter. Indeed, some studies provide evidences for the positive influence of plants on the dispersal and activity in soil of motile pollutant-degrading microbial inoculants. For example, in greenhouse experiments Fernandez-Lopez et al. (2021) observed an increase in transport and cometabolism of passively dosed ^{14}C-pyrene in pots planted with sunflowers (plants with a proven phytoremediation potential for PAH-polluted environments) and inoculated with the motile soil bacterium *P. putida* G7, as compared with unplanted pots. Later on, another greenhouse study examined ways for reducing the risk from bioaugmentation with this motile bacterium, through the trapping of pollutants and bacteria mobilized into the pore-water through plant-biochar arrangements (Castilla-Alcantara et al., 2023a). The compounds γ-aminobutyric acid (GABA), fructose, and citrate were identified as the sunflower root exudate components that triggered the strongest chemotactic reaction in *P. putid*a G7 cells, facilitating, in the absence of hydraulic flow, their penetration in pores of sizes on the same order of magnitude of the cell size (Castilla-Alcantara et al., 2022), and the access and activity at distantly located pollutant sources in a model aquifer (Castilla-Alcantara et al., 2023b).

Bacteria with plant-growth promotion (PGP) properties, can mitigate plant responses to stress, and enhance plant growth and development on contaminated substrates (Ahmad et al., 2018). PGP inoculants may act by a variety of mechanisms. They may act as biofertilisers (by increasing the availability of essential nutrients through *e. g.*, N_2 fixation and phosphate and iron solubilisation); organic contaminant degraders (lowering both

674 contaminant phytotoxicity and evapotranspiration); phytostimulants (producing plant
675 growth regulators and hormones, such as indoleacetic acid -IAA-, cytokinins and other
676 auxins); stress controllers (by decreasing ethylene production through the synthesis of 1-
677 aminocyclopropane-1-carboxylic acid deaminase -ACCD-); and as plant defence
678 inducers against phytopathogens (by producing siderophores, antibiotics, or fungicidal
679 compounds) (Saeed et al., 2021). Further improvements in the partnership between PGP
680 bacteria and plants may be obtained by the introduction of biochar as carrier for bacterial
681 inoculants (Xiang et al., 2022). Apart from improving bacterial survival and colonisation
682 in soil, biochar immobilise pollutants in the environment decreasing their bioavailability
683 and toxicity to plants and microorganisms, and provides non-contaminant organic carbon
684 to the soil. Hussain et al., (2018) provided evidence of the beneficial contributions of this
685 biochar–bacterium–plant synergy during rhizoremediation of soils contaminated by
686 petroleum hydrocarbons. These synergistic effects were further demonstrated during the
687 remediation of soil co-contaminated with heavy metals and PAHs using six plant species
688 and the same number of PAH-degrading bacterial strains isolated from oil-contaminated
689 soil (Sarma et al., 2019). The combined approach improved plant antioxidative defences
690 and helped to remove heavy metals and PAHs from the soil.

691 Therefore, having PGP features is also a positive trait for inoculants that may be
692 interesting in the case of phytoremediation procedures. In this type of procedures, and
693 specially in rhizoremediation, a good development of the plant root system is required in
694 order to achieve an adequate microbial activity. Beside plants, other soil meso- and
695 macrobiota may have positive influences on microbial degradation of organic pollutants.
696 Some studies have demonstrated that the metabolic activity of earthworms may enhance
697 the bioavailability of organic contaminants bioaccumulated in their tissues or released
698 through their casts, becoming bioavailable for the other organisms (Liu et al., 2015; Zhao

et al., 2016). Besides other compounds, such as carboxylic and amino acids, carbohydrates, polysaccharides, or proteins, plants can be able to exude enzymes (peroxidases, proteases, laccases, hydrolases, lipases, etc.) which may be involved in the degradation of organic xenobiotics (Dubrovskaya et al., 2017; Košnář et al., 2019). Those enzymes may break down some complex xenobiotics, making the metabolites more available for microbial mineralization.

8. Concluding remarks

Microbial inoculants are often used as unspecific components during soil bioremediation to reduce execution times, or when restrictions to microbial activity are suspected due to an initially low microbial biomass or toxicity stress. Despite the apparent simplicity of the technique, the effectiveness of bioaugmentation is difficult to predict and field applications often do not give the expected outcomes. The state-of-the-art in bioaugmentation provided in this chapter offers scientific foundations for a more efficient application of this technology in the clean-up of soils contaminated by organic pollutants. The introduced microorganisms should not only be selected on the basis of the desired catabolic capacities, but they should also be ecologically robust and competitive in front of native communities, and be able to disperse through soil. Microbial cooperation can constitute a further improvement to facilitate the establishment of inoculants in the soil, by exploiting synergistic catabolic functionalities, cross-feeding relationships, and facilitated microbial co-dispersion within microbial networks. Engineering components for an increased bioaugmentation performance include the immobilisation of the inoculants in materials such as biochar and clay minerals, serving as platforms for an enhanced microbial activity in soil, and the effect of plant roots through their physical

and chemical promotion of inoculant transport and activity, including the positive effects on microbial metabolism and tactic behaviour.

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1287 **Table 1.** Examples of bioaugmentation studies in soils contaminated with organic

1288 xenobiotics (2017-2023).

Inoculants	Contaminant	Concentration (mg kg^{-1})	Experimental conditions	Degradation efficiency	Reference
***Paracoccus aminovorans* HPD-2 (10^7-10^8 CFU g^{-1} of soil)**	PAH	452.76	*In vitro* microcosms, 20 g of soil (60% WHC)	~42% removal, after incubation for 42 d	(Wang et al., 2023)
Consortium of indigenous bacterial strains selected from soil (10^8 CFU g^{-1} of soil), including *Acetobacter, Achromobacter, Comamonas,* and *Pseudomonas.*	Petroleum hydrocarbons (TPH)	863-12818	Laboratory scale mesocosms, 1 kg of soil, 15-20% WHC	86% after 90 d	(Guarino et al., 2017)
***Candida* VITJzN04**	Lindane	100	Greenhouse pot experiments with garden soil	78% after 30 d	(Salam et al., 2017)
***Rhodococcus qingshengii* strain djl-6**	Carbendazim	2 - 8	Laboratory scale mesocosms (1 kg of soil, 15-20% water content)	> 93% after 14 d	(Chuang et al., 2021)
***Paenarthrobacter* sp. AT-5**	Atrazine	5	Laboratory scale microcosms (700 g soil, 30% WHC	95.9% after 7 d	(Jia et al., 2021)
Rhodococcus erythropolis IN101,* *Rhodococcus species IN306	Polychlorinated biphenyls (PCBs)	13	50 kg soil pile, temperature (17 – 25 o C), humidity (20 – 25%), pH (7.5 – 7.8), biogenic substances (N: P = 10:1)	87.5 after 6 months	(Wojtowicz and Steliga, 2020)
Mycobacterium dioxanotrophicus	1,4 dioxane	10 mg L^{-1}	Bioaugmented poplar	<4 µg/L in 13 days	(Simmer et al., 2020)

PH-06, Pseudonocardia dioxanivorans CB1190			rhizosphere. Poplar cuttings grown in plastic bins (25″ × 18″ × 7″) containing 20 L of solution		
Enriched DEHP-degrading consortium (*Rhodococcus* 30%) (10^9CFU g^{-1})	Spiked Di(2-ethylhexyl)phthalate (DEHP)	100 (0.35 native DEHP)	Microcosms of native contaminated soil+biochar (5%)(50% WHC)	87.5% in 42d *vs* 49.3% non bioaugmented control)	(Bai et al., 2020)
Enriched TBZ-degrading consortium *(Shingomonas* + *Hydrogenophaga* >20%*)*	Thiabenzadole	12, 250, 400	170g of native contaminated soil microcosms (40% WHC)	DT_{90s} of 8.5, 28.7, 33.9 (*vs* >250 in non bioaugmented controls)	(Papadopoulou et al., 2018)
Bacillus firmus PheN7 (**10^8 CFU g^{-1} of soil*)***	Spiked Phe (+ native PAHs	93.7 (19.4 native PAHs)	Anaerobic microcosms, 30 g of soil (30% WHC) with added NO_3Na (200 mM)	99% removal in 56d (*vs* 84% non bioaugmented controls)	(Zhou et al., 2022)

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Figure 1. Overview of factors affecting the performance of microbial inoculants employed in bioaugmentation of polluted soils.