
Strategies to Increase Bioavailability and Uptake of Hydrocarbons

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Abstract

The biodegradation of hydrocarbons in the environment is often slow due to restricted bioavailability. Research performed during the last 20 years has shown possible pathways to increase the bioavailability of hydrocarbons without necessarily increasing the risk to the environment. Pollutant solubilization through (bio)surfactants, microbial transport, and attachment to pollutant interfaces can increase bioavailability, which translates into an enhancement of biodegradation rates. These strategies can not only be integrated into optimized bioremediation protocols that lead to lower decontamination endpoints in soils and sediments but also help to improve biodegradation in other environmental contexts, such as wastewater treatment and natural attenuation.

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1 Introduction

With our deep knowledge of how hydrocarbons undergo biodegradation in the environment, it is not surprising that the application of biological technologies to the treatment of hydrocarbon pollution is well established. For example, of the 16 polycyclic aromatic hydrocarbons (PAHs) listed by the US Environmental Protection Agency (US-EPA) as priority pollutants, those with a molecular weight of up to 202 g/mol, including the high-molecular-weight PAHs pyrene and fluoranthene can be degraded microbially through growth-linked aerobic reactions, while the rest of the PAHs in that list, such as benzo[a]pyrene, are susceptible to cometabolic removal (Niqui-Arroyo et al. 2011). These reactions are the basis of a variety of approaches applied to biologically treated soils contaminated with hydrocarbons, which include landfarming, composting, bioreactor treatments, and phytoremediation (Ortega-Calvo et al. 2013). However, the benefits derived from the biodegradation of these chemicals are also accompanied by the uncertainties that surround the use of biological treatment when the bioavailability of these chemicals remains unpredictable. The current poor predictability of endpoints associated with the bioremediation of hydrocarbons is a large limitation when evaluating its viability for treating contaminated soils and sediments.

Three questions must be addressed when incorporating bioavailability science into bioremediation: (1) What is meant by “bioavailability?” (2) How should it be measured? and (3) Is it possible to increase bioavailability without enhancing environmental risks of the pollutants? In this chapter, we examine from an environmental perspective the potential of bioavailability-promoting strategies to enhance the microbial biodegradation of hydrocarbons. Therefore, the focus will be on mechanisms that eventually allow the integration of a bioavailability-efficient technology into current bioremediation practices.

2 The State of the Art in Bioavailability Science

2.1 The Bioavailability Concept

Over the last 30 years, numerous publications have discussed the concepts and definitions of bioavailability of organic chemicals. These have been summarized recently in the context of risk assessment and regulation (Ortega-Calvo et al. 2015) and are illustrated in Fig. 1. The main schools of thought consider bioavailability (focusing on the aqueous or dissolved contaminant), bioaccessibility (incorporating the rapidly desorbing contaminant in the exposure), and chemical activity (determining the potential of the dissolved contaminant for biological effects). Using the same framework, the figure places these different schools (Ehlers and Luthy 2003; Semple et al. 2004; Reichenberg and Mayer 2006) that have dissected bioavailability into the different processes that are involved (A to E), the dissimilar endpoints (bioaccessibility and chemical activity), and the different methodologies (desorption

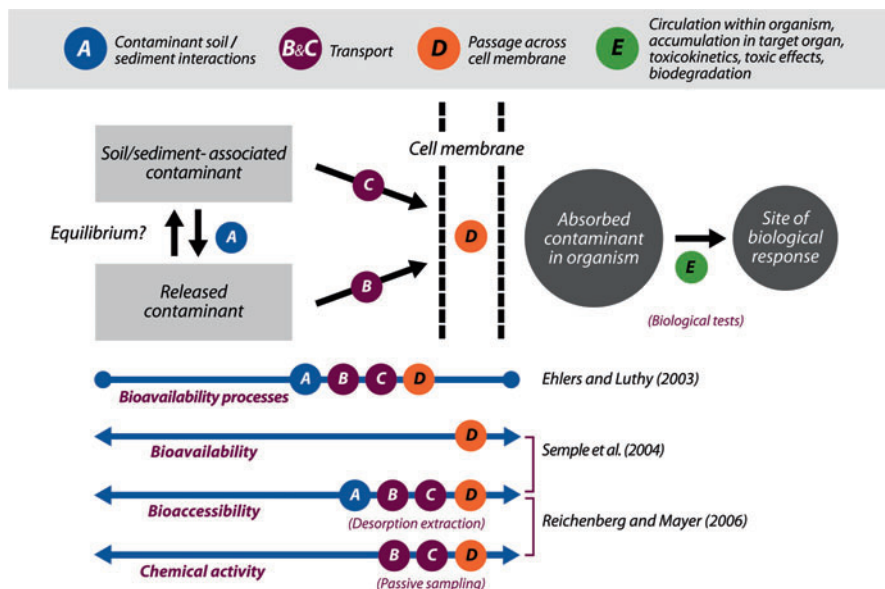


Fig. 1 Overview of scientific concepts of the bioavailability of organic chemicals (Reproduced with permission from Ortega-Calvo et al. 2015)

extraction, passive sampling, and biological tests). Each of these processes, end-points, and methods has been considered differently in a wide variety of bioavailability scenarios. Depending on the processes investigated, bioavailability can be examined through chemical activity (by including the processes B, C, and D) or bioaccessibility measurements which incorporate to B–D the time-dependent release of the contaminant from the soil/sediment. Depending on the biological complexity, the passage of the contaminant molecule across the cell membrane (process D) may represent multiple stages within a given organism before the site of biological response is reached (process E).

Chemical and biological approaches can be used to measure the bioavailability of organic chemicals. The results of infinite sink methods using Tenax and cyclodextrin extraction are currently used to predict toxicity and biodegradation and are in the process of being standardized. The results of these methods represent and define what is referred to as the rapidly desorbing fraction. The second complementary approach is the use of passive sampling to determine the freely dissolved concentration as a measure of the chemical activity of organic chemicals in soils and sediments. This approach proposes that chemical activity drives bioavailability (Fig. 1). Finally, several (mostly standardized by the International Organization for Standardization and the Organization for Economic Cooperation and Development) ecotoxicological test methods are available to determine bioavailability in the soil and sediment compartments to invertebrates, plants, and microorganisms (Ortega-Calvo et al. 2015).

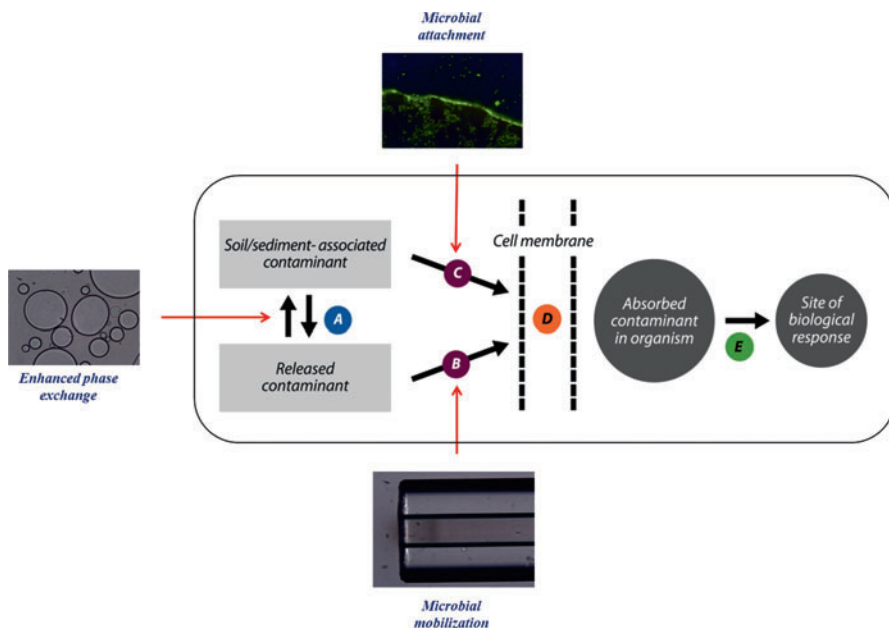


Fig. 2 Strategies to influence bioavailability processes (A to E, as described in Fig. 1), in connection with the microbial degradation of hydrocarbons

2.2 Strategies to Enhance Bioavailability for Biodegradation

Several strategies can act on bioavailability processes to enhance the microbial degradation of hydrocarbons (Fig. 2). Enhanced phase exchange (represented in the figure by the surfactant action – process A), microbial mobilization (represented by a band of chemotactic bacteria attracted by a chemical gradient inside a capillary – process B), and attachment to interfaces, that allows the direct acquisition of the soil/sediment-associated contaminant (process C), can increase bioavailability to hydrocarbon-degrading microorganisms and, therefore, biodegradation. In Fig. 2, process D represents the pollutant uptake by the microbial cells, necessary for biodegradation processing (process E), what may involve the mineralization of the chemical and the incorporation of substrate carbon into biochemical components and metabolites.

In a bioremediation context, these strategies should be integrated in such a way that the risks to the environment are minimized, such as the modulation of pollutant release relative to the actual biodegradation potential; the use of environmentally acceptable agents, such as (bio)surfactants, microorganisms, and plants; and the application of treatment methods requiring minimal handling of the polluted soil or sediment. A conservative approach is needed, because in some circumstances, bioremediation may even increase the risks of the pollutants (Ortega-Calvo et al. 2013). Polluted soils and sediments treated by bioremediation may increase their

toxic potential, due to the release of sorbed hydrocarbons and the formation of toxic metabolites, although prolonged treatments may help to minimize these risks. This knowledge has also implications in other environmental contexts where the lack of biodegradation plays a relevant role in the persistence of hydrocarbons, such as wastewater treatment and natural attenuation (Niqui-Arroyo et al. 2011).

3 Enhanced Pollutant Phase Exchange

The positive effects of promoting the phase exchange of hydrocarbons on their biodegradation in soil have been known for some time (Ortega-Calvo et al. 1995). For example, surfactants can increase the biodegradation rates of hydrocarbons that desorb slowly from soils (process A in Fig. 2). In general, nonionic surfactants are the most frequently used types of surfactants in biodegradation studies, mainly because they are uncharged, which minimizes any eventual toxic effects (Martin et al. 2014). In a study focused on the bioavailability of slow desorption hydrocarbons, the nonionic surfactant Brij 35 was applied to a soil originating directly from a site polluted by creosote and a soil from a manufactured gas plant (MGP) that had been treated by bioremediation (Bueno-Montes et al. 2011). In the creosote-polluted soil, biodegradation was inhibited by the surfactant, but biodegradation in the bioremediated MGP soil, which was enriched in slow desorption hydrocarbons, was enhanced. The different outcomes were likely a consequence of the balance of two effects, namely an increase in the bioaccessibility of the chemicals and an enhancement of the consumption rate of other PAHs present in the soil at the same time, yielding subsequent competition effects. Therefore, the selective use of surfactants in soils enriched in hydrocarbons that desorb slowly would not only avoid the inhibition of biodegradation through competition mechanisms. This strategy would also allow for the reduction of risks associated with an increased chemical activity and toxicity of the hydrocarbons and metabolites as a result of solubilization, at concentrations in excess of the metabolic potential of the microorganisms. Other advantages of this different way of surfactant action may relate to the biodegradable nature of the surfactant, which is obviously necessary to minimize environmental impacts. The use of a surfactant involves eventual increases in nutrient and oxygen demands, which would be less important when pollutant loads are attenuated and less bioaccessible, as well as the decrease in concentration of surfactant after extended biodegradation periods, when it is required to solubilize the slow desorption hydrocarbons. However, the use of surfactants in environmental remediation may be potentially risky because they may be toxic and diminish the soil quality, leading to the need for other economic considerations. As a result, it may potentially be better to use biosurfactants or other naturally occurring stimulants.

Electrokinetics can also be successfully employed to enhance the bioavailability of hydrocarbons, particularly for the surfactant-assisted bioremediation of soils that are rich in clay fractions and/or aged contaminants (Niqui-Arroyo et al. 2006; Niqui-Arroyo and Ortega-Calvo 2007, 2010). These studies made use of the surfactant Brij

35 to optimize the process performance. The residual concentrations of the total biodegradable PAHs remaining after bioremediation in soil slurries were twofold lower in electrokinetically pretreated soils than in untreated soils (Niqui-Arroyo and Ortega-Calvo 2010). It is conceivable that physicochemical changes produced in polluted soil particles that were exposed to electric fields may promote the bioavailability of these PAHs, thus improving the bioremediation performance. The application of an electric field to the soil in the presence of the surfactant, through the electroosmotic flow that was consequently generated, enhanced the bioavailability of the PAHs by changing their desorption kinetics. The development of an electroosmotic flow through soil aggregates could probably have caused an increase in the rate of slow desorption (Shi et al. 2008). The induced physical and chemical changes could have resulted in the mobility of the pollutant fraction entrapped within the soil nanopores and/or strongly sorbed to black carbon.

Microbial and plant biosurfactants, if properly managed, are also able to improve bioremediation performance through an enhanced phase exchange. Many different surface-active compounds synthesized by a wide variety of microorganisms, such as *Pseudomonas*, *Bacillus*, *Acinetobacter*, and *Mycobacterium*, have been identified (Banat et al. 2010). The exact physiological roles of microbial surfactants are apparently not restricted to the solubilization of hydrophobic carbon sources, as surfactants can also be produced when the microorganisms are grown with water-soluble substrates, such as glucose. These biosurfactants are important in a number of ecological processes and have been linked to microbial adhesion, antagonistic effects toward other microorganisms, heavy metal sequestration, and cell-cell communication. Although the production of biosurfactants is not universal among all microbes, their effect on the bioavailability of hydrocarbons in the natural environment causes these agents to be an important factor that should be considered when optimizing bioremediation. Indeed, rhamnolipid biosurfactants can dissolve pure, solid PAHs, such as phenanthrene, thus increasing their rate of biodegradation (Garcia-Junco et al. 2001, 2003; Resina-Pelfort et al. 2003), and enhance the desorption and biodegradation of slowly desorbing and aged pyrene present in soil (Congiu and Ortega-Calvo 2014; Congiu et al. 2015).

In addition to biosurfactants, there is a wide variety of other natural organic compounds derived from either microbes or plants that can potentially increase the bioavailability of hydrocarbons. For example, cyclodextrins (Garon et al. 2004) and unsaturated fatty acids (Yi and Crowley 2007) have been proposed to stimulate the biodegradation of PAHs in soil through this mechanism. The bioavailability of hydrocarbons can be enhanced by the general capacity of dissolved organic matter (DOM) to mobilize hydrophobic chemicals (Tejeda-Agredano et al. 2013). This enhancement can occur through a variety of process: (1) enhanced desorption (Haderlein et al. 2001), (2) direct access to DOM-sorbed PAHs due to the physical association of bacteria and DOM (Ortega-Calvo and Saiz-Jimenez 1998), and (3) increased diffusional flux through unstirred boundary layers around bacterial cells (Haftka et al. 2008), similarly to the enhanced uptake of cadmium by spinach plantlets originated from seed germination in the presence of labile metal complexes (Degryse et al. 2006). However, DOM can also inhibit biodegradation in exposure

scenarios governed by a low chemical activity of the pollutants, by preventing the bacterial attachment to the hydrocarbon source (Tejeda-Agredano et al. 2014).

4 Microbial Mobilization

The bioavailability of hydrocarbons can be increased not only by mobilizing the pollutants but also by promoting the dispersal of microorganisms throughout the polluted matrix, to have a better access to the released contaminant (process B in Fig. 2). However, movement of pollutant-degrading microorganisms in porous media is often restricted by their high deposition rates and adhesion to soil surfaces (Ortega-Calvo et al. 1999; Lahlou et al. 2000). To this end, chemotactic mobilization of flagellated bacteria has gained attention in bioremediation (Marx and Aitken 2000; Krell et al. 2013). A technological innovation based on this concept makes use of the mobilization potential of pollutant-degrading microorganisms from rhizospheres, with chemotactic responses demonstrated toward moderately hydrophobic PAHs, such as phenanthrene, anthracene, and pyrene, and are able to move chemotactically at speeds of approximately 1 mm/min (Ortega-Calvo et al. 2003). It has also been shown in later research that motility and transport of hydrocarbon-degrading bacteria can be controlled through a suitable choice of chemical effectors, including carbon sources and nanomaterials (Velasco-Casal et al. 2008; Ortega-Calvo et al. 2011; Jimenez-Sanchez et al. 2012, 2015). In well-controlled column systems, we assessed the influence of different effectors on the deposition of a chemotactic, naphthalene-degrading bacterial species, *Pseudomonas putida* G7, in selected porous environments (sand, forest soil, and clay aggregates). Cellular deposition, however, was concomitantly dependent on the cellular motility (hypermotility, attraction, or repulsion), the sorption of the effector to the column packing material, and the resulting pore-water concentration. For example, an exposure of the cells to salicylate and naphthalene induced a smooth movement with few acceleration events and positive taxis, while cells exposed to silver nanoparticles (AgNPs) exhibited tortuous movement and repulsion. Although glucose was metabolized by strain G7, it did not cause any attraction, but it induced the cells to go into a hypermotile mode, characterized by a high frequency of acceleration events, a high swimming speed ($>60 \mu\text{m s}^{-1}$), and a high tortuosity in the trajectories. The chemically induced motility behaviors demonstrated a distinct affinity of cells for sand particles in batch assays, resulting in the development of breakthrough curves in percolation column experiments. Salicylate and naphthalene significantly reduced the deposition of G7 cells in the column experiments, while glucose and AgNPs enhanced the attachment and caused a blocking of the filter, which resulted in a progressive decrease in deposition. This work was later extended to show distinctive tactic behaviors and mobilization as a result from exposure to zero-valent iron nanoparticles (Ortega-Calvo et al. 2016) and chemoeffector-containing DOM (Jimenez-Sanchez et al. 2015).

Another approach for facilitated microbial dispersal, in connection with hydrocarbon-polluted scenarios, is based on bacterial/oomycete interactions

(Sungthong et al. 2015, 2016). Our results show that zoospores can act as ecological amplifiers of fungal and oomycete actions extending the concept of “mycelial pathways” for dispersal of pollutant-degrading bacteria (Kohlmeier et al. 2005; Furuno et al. 2010). This strategy may be of relevance for nonflagellated bacterial PAH degraders, such as *Mycobacterium* species, which may constitute a significant fraction of the functional microbiome in PAH-polluted environments (Uyttebroek et al. 2006a). Although these bacteria seem to be less well transported through mycelial pathways than self-propelled bacteria (Kohlmeier et al. 2005), the absence of motility is, in relative terms, a positive factor for the biomobilization caused by zoospores. This can be explained by the link of this mobilization mode with flow dynamics. The thrust force created by zoospore swimming mobilizes more efficiently immotile bacteria than actively swimming bacteria. Besides, flagellated (and therefore chemotactically active) bacterial groups, such as *Pseudomonas* and *Achromobacter*, can be dispersed through their own chemotactic navigation along mycelial pathways (Furuno et al. 2010), but they could also be biomobilized by zoospores at the cell growth phases when flagellar motility is limited or not existing.

5 Microbial Attachment

Microorganisms can also increase the bioavailability of hydrocarbons when in direct contact with the pollutant source through attachment (Fig. 2, process C), thereby enabling biodegradation to occur, or even to proceed more rapidly (Ortega-Calvo and Alexander 1994; Garcia-Junco et al. 2003; Uyttebroek et al. 2006b; Tejada-Agredano et al. 2011; Zhang et al. 2016). A feasible strategy to enhance bioavailability is, therefore, to promote the growth and activity of attached bacteria. The main goal of the study focused on PAHs present in nonaqueous-phase liquids or NAPLs (Tejada-Agredano et al. 2011) was to target the potential nutritional limitations of microorganisms to enhance the biodegradation of PAHs at the interface between the NAPL and the water phase. The results indicated that the biodegradation of PAHs by bacterial cells attached to NAPLs can be limited by nutrient availability as a result of the simultaneous consumption of PAHs within the NAPLs, but this limitation can be overcome by the addition of an oleophilic biostimulant that act as an interface fertilizer, by providing nutrients to attached bacteria, thus promoting bioavailability.

Bacteria attached to hydrocarbon/water interfaces can access to, theoretically unaccessible, submicrometer pores through proliferation and penetration (Akbari and Ghoshal 2015; Akbari et al. 2016). In a series of studies with the hexadecane-degrading, immotile bacterium *Dietzia maris*, Ghoshal et al. determined that the hydrocarbon remained non-bioavailable when it was separated from bacteria by membranes with pores that were 3 μm or smaller. Given the size of individual cells (0.9 μm), their strong tendency to form aggregates (6.0 μm), and the need for attachment to the hydrocarbon phase as a prerequisite for biodegradation of this sparingly soluble compound, the cause for this poor bioavailability was the lack of

physical contact between the hydrocarbon phase and the bacterial aggregates (Akbari and Ghoshal 2015). However, during growth in hexadecane-wetted membranes, morphological changes of attached cells allowed the penetration in the membranes through pores that were smaller than the cell size (Akbari et al. 2016). Authors postulated that this penetration was caused by physical forces imposed by growth, colony extension, and biosurfactant production. In this way, bacterial translocation and passage through the pores allowed the mineralization of ^{14}C -labeled hexadecane that was separated from the filter by an aqueous phase. Therefore, bacterial plasticity may not only play a role in the attachment and colonization of distant, separate phase hydrocarbons located in submicron porosities (process C in Fig. 2) but also in the biodegradation of the dissolved fractions of the chemicals which are only available through diffusion and transport through these small pores (process B).

6 Research Needs

Bioavailability was recognized as a research priority in bioremediation as early as 25 years ago (Alexander 1991). Over these years, significant advancements have been made to understand how pollutant phase exchange, microbial mobilization, and cell attachment to interfaces affects bioavailability during biodegradation of organic chemicals and, particularly, hydrocarbons. In spite of these advancements, significant gaps of knowledge exist between bioavailability science and bioremediation. Still today, it is difficult to predict bioavailability of hydrocarbons, for example, solely on the basis of basic parameters such as organic matter, black carbon or clay contents of a given soil or sediment, and the physicochemical constants of the chemicals (such as solubility in water and octanol-water or organic-carbon based distribution coefficients). This limitation even remains with improved assessments through determinations of chemical activity and bioaccessibility. This uncertainty not only applies to biodegradability in natural environments but also to engineered systems where bioavailability-promoting strategies are implemented. Therefore, research is needed to provide a solid link among chemical activity, bioaccessibility, and biodegradation of hydrocarbons. A main research need is also to understand how biological networks react in situ to sustainable, bioavailability-promoting approaches involving biostimulants, specialized microorganisms, and plants for an enhanced pollutant carbon turnover. The findings achieved with simple laboratory or microcosm systems need to be translated to field operations, and this scale-up faces serious difficulties, given the complexity of intra- and interspecific biological interactions occurring in polluted sites. The assessment of possible risks, such as increases in compound toxicity due to bioremediation, should be also part of future research.

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