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ABSTRACT

Petroleum hydrocarbons and toxic metals are sources of environmental contamination and are harmful to all ecosystems. Fungi have metabolic and morphological plasticity that turn them into potential prototypes for technological development in biological remediation of these contaminants due to their ability to interact with a specific contaminant and/or produced metabolites. Although fungal bioinoculants producing enzymes, biosurfactants, polymers, pigments and organic acids have potential to be protagonists in mycoremediation of hydrocarbons and toxic metals, they can still be only adjuvants together with bacteria, microalgae, plants or animals in such processes. However, the sudden accelerated development of emerging technologies related to the use of potential fungal bioproducts such as bioinoculants, enzymes and biosurfactants in the remediation of these contaminants, has boosted fungal bioprocesses to achieve higher performance and possible real applications. In this review, we explore scientific and technological advances in bioprocesses related to the production and/or application of these potential fungal bioproducts when used in remediation of hydrocarbons and toxic metals from an integral perspective of biotechnological process development. In turn, it sheds light to overcome existing technological limitations or enable new experimental designs in the remediation of these and other emerging contaminants.

Keywords: Mycoremediation, Contamination, Bioinoculants, Enzymes, Biosurfactants

Abbreviations

PAH, polycyclic aromatic hydrocarbons; AMF, arbuscular mycorrhizal fungi; NO_3^-, nitrate; SO_4^-, sulfate; CO_2, carbon dioxide; H_2O, water; GC-MS, mass spectrometry coupled to gas chromatography; CYP, cytochrome P450 monooxygenases; Eh, redox potential; HBT, 1-hydroxybenzotriazole; ABTS, 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); ROS, reactive oxygen species; NER, non-extractable residues; CMC, critical micelle concentration (CMC); SL, sophorolipids; MEL, mannosylerythritol lipids; PL, polyol lipids; RBBR, Remazol Brilliant Blue R; CTAB, cetyltrimethylammonium bromide; DCPIP, 2,6-dichlorophenol indophenol; CG, gas chromatography; MIC, minimum inhibitory concentration; AAS, atomic absorption spectrometry; ITS, Internal Transcript Spacer; DGGE, denaturing gradient gel electrophoresis; FISH, fluorescence *in situ* hybridization ; T-RFLP, terminal restriction fragment length polymorphism; STR, stirred tank reactors; $CaCO_3$, calcium carbonate; $Na_5P_3O_{10}$, sodium tripolyphosphate.

1. INTRODUCTION

Environmental contamination by petroleum hydrocarbons and toxic metals is a worldwide problem that threatens public health. Petroleum industrial activities, anthropogenic actions and environmental disasters are frequent and represents major sources for such contamination [1–3]. Petroleum is a recalcitrant compound with a complex composition of saturated, unsaturated and ramified hydrocarbons, which includes aliphatic, alicyclic, monoaromatic, polycyclic aromatic hydrocarbons (PAHs), resins, and asphaltenes [4,5]. Aliphatics have lower toxicity than aromatics (alkylbenzenes, PAH) and alicyclics. Low molecular weight PAHs have up to 3 fused benzene rings, while high molecular weight PAHs have 4 rings or more (Fig. 1) [5]. The volatility of these hydrocarbons decreases with increasing molecular weight and exhibit low solubility and availability, especially in long-term contaminated soils [6]. In living cells some metals such as cobalt (Co), copper (Cu), iron (Fe), magnesium (Mg), manganese (Mn), molybdenum (Mo), selenium (Se), and zinc (Zn) are essential for various biochemical functions in appropriate concentrations, but at high concentrations these metals are toxic. Other non-essential metals such as arsenic (As), lead (Pb), chromium (Cr), mercury (Hg), and cadmium (Cd) are toxic even at very low concentrations [7,8].

Fig. 1 Chemical structures of the principal petroleum hydrocarbons: aliphatics (a), alicyclics (b) and aromatics (c)

In this context, hydrocarbons and toxic metals exhibit cytotoxic, carcinogenic and mutagenic effects, which tend to decrease the biotic potential in terrestrial and aquatic ecosystems [9]. These contaminants can bioaccumulate in animal or plant cells [2,10], and particularly metals

have the potential to be transported through the food chain undergoing biomagnification [3]. Thus, there is an urgent need for the development of technologies to remediate hydrocarbons and toxic metals.

Biological remediation is an eco-sustainable and more cost-effective technology compared to physical or chemical treatments of hydrocarbons and toxic metals [11,12]. Patents and articles on biological remediation reported between 1997-2017 point out that the main technologies available are those intended for the remediation of hydrocarbons (38%) and toxic metals (21%) in soils [12]. Furthermore, the number of articles and patents on fungi as bioremediation agents is only surpassed by those based on bacteria [12]. This specific fact can be partially explained by the 30-year gap for such purposes between the initiation of fungal bioprocesses (1970s) compared to bacteria (1940s) [13,14]. Furthermore, technological development involving the production and/or use of fungal metabolites for such specific purposes has only begun in the past 30 years (in 1990s) [15]. Thus, it is presumed that although fungi of various morphologies such as arbuscular mycorrhizal (AMF) [16], filamentous [17,18], unicellular yeast [19,20] or mushrooms [21,22] have been reported as promising prototypes for technological development in hydrocarbon and toxic metal remediation, they still remain underexplored for such purposes.

Prototypes, in technological development of services or products, are structures projected as a proof concepts to validate their feasibility, verify their performance, and collect feedback that may enhance their features before making them commercially available [23]. In this context, fungi are prototypical service providers in remediation due to their ability to assimilate hydrocarbons and obtain energy or produce biomass through passive mechanism (without energy expenditure), as well as toxic metals interaction (e.g., biosorption) [24–26], which in turn also makes them product providers as (bio)-inoculants for mycoremediation. Alternatively, through active metabolism, fungi are also prototype providers of potential valuable metabolites used in mycoremediation, which includes enzymes [27], biosurfactants [28], organic acids [29], polymers [16], pigments [30] and siderophores [31]. Fungal strains produce these metabolites to promote their survival, growth and protection in diverse environments. However, this ecological potential to colonize and/or remediate environments can be enhanced when the use of these prototypes is exploited through biotechnological processes [8,32].

The potential of fungi belonging to the phyla Ascomycota [33,34], Basidiomycota [21,35], Glomeromycota [36,37], and Mucoromycotina (*incertae sedis*) [18,38] to remediate hydrocarbons and toxic metals arises from their ability to thrive in such environments and the capability to engage in natural attenuation processes. However, higher performance compared to natural attenuation can be achieved when the biotic potential of these fungal strains is increased through changes in physicochemical parameters (pH, moisture and aeration) at the site and/or by

the addition of nutrients to favor biostimulation of the indigenous microbiota [39,40]. Furthermore, these autochthonous fungi can be isolated, domesticated and also used as bioinoculants to carry out bioaugmentation of the microbiota involved in remediation through passive or active mechanism [41,42]. In other words, fungi bioprospecting allows the selection of those that have potential to be used to produce bioinoculants or metabolites applied to environmental remediation [43,44]. Bioinoculants are selected strains that resist the toxicity and presence of other microorganisms in the contaminated site and still have the ability to interact with contaminants and/or produce metabolites for remediation [28,41]. These metabolites can be different biochemical molecules that interact with contaminants to carry out or enhance the steps involved in environmental decontamination [17,30].

The degradation pathways and interaction of fungal metabolites with hydrocarbons and toxic metals are sources of speculation that enable more assertive bioproduct development [24,31]. Biotechnological processes are under constant development to enable large-scale production of enzymes and biosurfactants, but the market for these fungal bioproducts to remediate hydrocarbons and toxic metals is still incipient [45,46]. Only fungal biosurfactants are already marketed for this purpose [47,48]. The main obstacle to the practical application of products containing enzymes and/or bioinoculants as metabolite producers is related, respectively, to the sensitivity to environmental and nutritional parameters of these products in contaminated environments [1,49]. The high costs, low productivity and technological limitations related to the production/application of fungal bioproducts also still restrict their use [1,45].

Biotechnology companies and universities worldwide have boosted fungal prototyping from the development of emerging technologies to propose, elucidate and/or enhance bioprocesses focused on the containment/removal/degradation/detoxification of hydrocarbons or toxic metals [45,50,51]. These emerging technologies are the result of technical-scientific advances and innovations in various fields that promote “biotic and abiotic resource optimization” or “performance enhancement” in various steps of fungal bioprocesses focused on environmental remediation [32,52]. Figure 2 shows the main bioprocess fields in mycoremediation.

Fig 2. Main current insights in scientific-technical development from the use of fungi in mycoremediation

In this context, technological development is premised on exploring the biodiversity of fungal strains that allows the expansion of resources to: (1) elucidate mechanisms of action and degradation pathways [24,53]; (2) enhance existing technologies involved in process steps [32], (3) minimize negative effects related to abiotic and biotic parameters [31,41]; and (4) propose alternative strategies through physicochemical-biological systems or biosystems to add resources or overcome technological limitations [52,54]. In summary, such biotechnological resources serve as supports in mycoremediation processes and generally have as “starting point”

the bioprospecting in different environments, with a possible “end point” being the formulation of bioproducts and ecotoxicological tests to ensure environmental safety [17,23,35].

Although the mycoremediation of hydrocarbons and toxic metals is not a new subject in the scientific literature [39,40], its approach is promising and current, which corroborates with the high number of research articles proposing technologies and review articles presenting the scientific state of the art in different fields of knowledge [37,55,56]. No reviews, however, were found regarding to scientific advances and emerging technologies from a mechanistic perspective focused on the integral mycoremediation processes from bioinoculants, enzymes and biosurfactants. This gap, when overcame, can extrapolate the current interface between available and innovative technologies for fungal bioprocesses in hydrocarbon and toxic metal remediation. This review therefore aimed to compile and explore information on the main mechanistic actions and use of fungal bioproducts such as bioinoculants, enzymes and biosurfactants for the remediation of hydrocarbons and toxic metals. We also comprehensively scrutinized the scientific and technological advances to promote, enhance and increase the performance of these bioproducts when used for the remediation of such contaminants.

2. FUNGAL MECHANISMS AND ENZYMES FOR HYDROCARBONS REMEDIATION

Oxygen, the second most available element in air, is involved in natural hydrocarbon degradation processes, and acts as the final electron acceptor in aerobic microbial metabolism, which promotes higher energy production compared to anoxic conditions [57,58]. In general, fungal bioinoculants under oxygen-limited or anaerobic conditions (e.g., aquifers, sludge or mangroves) utilize various inorganic radicals or compounds such as nitrate (NO_3^-), sulfate (SO_4^-), Fe^{+2}, Mn^{+2} and carbon dioxide (CO_2) as final electron acceptors [11,57]. Anoxic conditions can also be generated during hydrocarbon degradation due to an increase in microbial respiration during the consumption of readily assimilable substrates used to support microbial growth [58]. Anaerobic degradation however, can occur at negligible rates and produce metabolites that are more toxic than their original counterpart and/or inhibit other strains during degradation [6,59].

One of the main ecological contributions of fungi is their ability to produce enzymes that cleave chemical bonds and/or transfer functional groups in different hydrocarbon structures. The catalysis involved in hydrocarbon degradation, besides providing energy for strains, assists the transfer of electrons from a reduced organic substrate (hydrocarbons-donor) to another chemical compound (acceptor) [17,59]. Although the same set of fungal enzymes partially or completely

degrade or detoxify hydrocarbons, the degradation pathways and mechanisms involved in these processes may be different for each hydrocarbon [4,60]. Susceptibility to hydrocarbon degradation by fungal enzymes can be classified by the following sequence: linear aliphatic> branched aliphatic> aromatic> alicyclic [5,61]. Possible hydrocarbon degradation pathways are deduced through combined analyses of genome annotation/transcriptomic and gas chromatography coupled to a mass spectrometry (GC-MS) profile [31,53]. Thus, possible metabolic pathways involved in degradation of aliphatic and alicyclic hydrocarbons, PAHs and alkylbenzene are shown in Figures 3 and 4, respectively.

Fig. 3 Possible peripheral pathways for degradation of aliphatic and alicyclic hydrocarbons by fungi are shown in **a** and **b**, respectively (modified from [4,5,62])

Fig. 4 Possible peripheral pathways for degradation of PAHs and alkyl-benzene by fungi are shown in **a** and **b**, respectively (modified from [4,60])

When incorporated within the fungal cell, hydrocarbons are degraded by intracellular enzymes via cytosolic or mitochondrial pathways [53,63]. Mass transfer of hydrocarbon into cells by a passive mechanism can be restricted to specific structural fractions of the molecule [64]. Hydrocarbon degradation and intracellular lipid metabolism is speculated in fungal cells, since hydrocarbons can be stored in lipid bodies and be oxidized simultaneously in neighboring peroxisomes [24,65]. Extracellular enzymes also partially degrade hydrocarbons, producing different intermediate metabolites that may be assimilated by the fungus itself or by other strains for further detoxification [53,60].

Intracellular enzymes such as epoxide hydrolases, quinone oxidoreductases and cytochrome P450 monooxygenases (CYPs) catalyze several reactions involved in hydrocarbon degradation, such as hydroxylation, epoxidation, dealkylation, sulfoxidation, deamination, desulfurization, dehalogenation and N-oxide reduction in fungal cell [57,63]. The number of CYPs genes can vary according to morphological characteristics of the fungus. Saccharomycotina yeasts (*Candida maltosa, C. tropicalis*, *C. apicola*, *Yarrowia lipolytica*) have relatively few CYPs genes, while filamentous Eurotiales fungi (*Aspergillus flavus*, *Monascus ruber* and *Penicillium chrysogenum*) tend to have high numbers of CYPs [11,66]. Other intracellular enzymes such as transferases (glutathione system), hydroxylases, dioxygenases, dehydrogenases, reductive dehalogenases, nitro-reductases and tyrosinases (sometimes extracellular) may also be involved in hydrocarbons degradation [31,53,63].

Extracellular enzymes such as peroxidases (lignin, manganese and versatile) and the (poly)-phenol oxidases (laccases, tyrosinases) have low substrate specificity, since they are involved in the degradation of lignin (composed of phenolic molecules), which also promotes their catalytic action in hydrocarbon degradation [63,67]. The redox potential (Eh) of these enzymes is directly related to hydrocarbon oxidation. Peroxidases have high Eh (1.0-1.5 V) and are dependent and

sensitive to H_2O_2 concentrations, while laccases have lower Eh (0.4-0.8 V) and can be produced by basidiomycetes and ascomycetes [68,69]. Although laccases and tyrosinases have catalytic similarities such as containing Cu in their structures and requiring O_2 for catalysis, fungal tyrosinases are still less studied for remediation of hydrocarbons compared to laccases [70,71]. Tyrosinases oxidize a smaller range of hydrocarbons than laccases and there is a risk of their irreversible inactivation during the degradation of these contaminants [72,73]. Additionally, hydrolytic enzymes such as lipases and esterases also promote hydrocarbon biotransformation, as genes encoding for these enzymes were expressed by *Dentipellis* sp. [69] and *Aspergillus sydowii* [53], respectively, during degradation of PAHs.

In summary, the reactions involved in enzymatic catalysis of hydrocarbons are aimed to initially incorporate molecular oxygen into their structure and produce more polar pre-intermediate metabolites, which also stimulates their subsequent degradation [5,57]. This goal is achieved through the formation/transfer of hydroxyl groups and/or cleavage of C-C bonds between adjacent phenolic hydroxyl groups of the hydrocarbon, which through specific metabolic pathways form other more soluble and less toxic intermediate metabolites, such as carboxylic acids and aldehydes [31,53]. These, in turn, can be excreted to be degraded by other microbial strains or enzymes; or are stored in lipid vesicles, or biotransformed by β-oxidation and the tricarboxylic acid cycle to produce simpler organic compounds such as pyruvate, acetate, and subsequently CO_2 and water (H_2O) [5,57,65].

2.1 Enzymatic mycoremediation

When enzyme-producing fungal bioinoculants are applied to contaminated soils, the produced enzymes diffusion is probably low, since excreted enzymes tend to concentrate near the cells and/or where substrates are available [74]. The practical use of enzyme-producing fungal bioinoculants for bioaugmentation in non-sterile contaminated soils may have their enzyme activity influenced due to microbial co-metabolism [60]. On the other hand, enzymatic mycoremediation occurs through the use of concentrated enzymes (crude or purified extract), and may present advantages compared to the use of bioinoculants [49,75]. This is corroborated by the fact that enzymes can exhibit: (1) higher catalytic activity; (2) smaller size, which improves their diffusion; and (3) higher stability and coverage over a wide range of physicochemical gradients and contaminant concentrations [27,49]. For instance, the use of crude laccases extract (60 $U \bullet mL^{-1}$) produced by *Pleurotus sajor-caju* at pH 3.2 and 30 °C promoted 55% removal of phenol (3.0 $mmol \bullet L^{-1}$) from an aqueous solution, which represents a higher percentage value when compared to the use of this strain as bioinoculant [27].

Enzymatic mycoremediation of hydrocarbons is mainly related to the use of laccases that are already commercially available [76,77]. Although enzymatic degradation of hydrocarbons is more easily carried out in liquid culture compared to the same strains growing in soil, studies of enzymatic mycoremediation in soil microcosms have also shown promising results for hydrocarbon remediation [78,79]. In summary, higher efficiencies in enzymatic mycoremediation are achieved when parameters such as pH, temperature, enzyme loading and Eh are properly controlled [75,80].

Although fungal laccases show higher Eh than those of bacteria and plants, this potential is still low for oxidation of high molecular weight hydrocarbons [81]. Alternatively, mediating compounds (natural or synthetic) such as 1-hydroxybenzotriazole (HBT), 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), coumaric acid and ferulic acid can be added to the reaction medium, which are initially oxidized (by laccase) forming reactive oxygen species (ROS) that increase the Eh for oxidation of higher molecular weight hydrocarbons [79,82]. The oxidation of ABTS by laccases may be influenced by mediator concentration and water availability [75,80]; since greater degradation of benzo[a]pyrene was speculated to occur in aqueous solutions than in soil microcosms, both containing ABTS [80]. The practical use of enzymatic mediators, however, may be unfeasible due to high costs and possible increase in toxicity [78,79].

In soils natural phenolic compounds may act as redox mediators for laccases and possibly enhance enzymatic degradation of hydrocarbons [83]. However, laccases from *Trametes versicolor*, when adsorbed on natural soil minerals such as Fe and aluminum (Al), decreased their enzymatic activity. Catalytic activity however, was increased under acidic conditions [84], which exemplifies the impact of parameters in enzymatic mycoremediation. Laccases can also have their catalytic activity inhibited or be irreversibly inactivated due to oligomerization of the products formed from oxidation of PAHs such as quinone derivatives and phenolic compounds via self-coupling reactions [72,73]. Furthermore, enzymatic mycoremediation of solid matrices requires a mixing process to promote a great enzyme dispersion (mass transfer), since the restricted spatial location of the enzyme may differ with the distribution of contaminants [1,74].

Several technologies have been proposed to evaluate and/or promote enhancements in enzymatic mycoremediation. For example, the use of packed-bed bioreactors and intermittent feeding of laccases (every 5 days) achieved higher degradation rates of chrysene (78%) and benzo[a]pyrene (35%) in soils within 10 days, while a slowdown of this rate was maintained up to 35 days of mycoremediation [75]. In addition, organic solvents and chemical surfactants are used to increase the availability of hydrocarbons prior to enzymatic mycoremediation, although such chemicals at high concentrations may alter stability or lead to enzyme inhibition [50,80].

The conversion of anthracene to anthraquinone reached titers greater than 95% within 12 h when the laccases and HBT (mediator) system was added to surfactant Tween 80 [85]. Laccase can also cleave C-O and C=C bonds present in the chemical structure of Tween 80 promoting the formation of ROS such as RO- and ROO-, which contribute to hydrocarbon degradation [85].

Some factors such as fate of degraded hydrocarbons, changes in ecotoxicity and soil microbial community, are criteria that evaluate the feasibility for practical use of enzymatic mycoremediation [35,77]. Laccases from *T. versicolor* promoted a lower mineralization rate of anthracene, benzo[a]anthracene and benzo[a]pyrene in soils compared to disposal of these PAHs (covalently bound to organic matter) as non-extractable residues (NER) [76,77]. NERs also enable the detoxification of PAHs due to their recalcitrance to breakdown and transport in organic matter [86]. Furthermore, the action of these laccases altered bacterial diversity during mycoremediation of these soils; and increased the toxicity of those contaminated with anthracene, probably due to intermediate metabolites that form from reactions subsequent to the initial oxidation of anthracene [76].

Biotechnological enzyme production with environmental interest presents limitations in terms of low productivity and concentration steps [1,46]. The enzymatic mycoremediation also present limitations due to financial and technological constraints, since the costs (production-related, mediators, etc.), operational instability and non-recovery of these enzymes after their use hinder large-scale application [49,74]. Thus, alternative substrates for production and extraction of laccases [79,87]; protein engineering and computational simulation [88,89]; use of natural mediators [90] and enzyme immobilization are strategies developed to overcome such limitations [73,82].

In this context, laccases extracted from spent mushrooms such as *Agaricus bisporus* and *Pleurotus eryngii* promoted degradation of anthracene, benzo[a]pyrene and benzo[a]anthracene between 66-100% in aqueous solution in 24 h [87]. Laccases from *T. versicolor* (expressed in *Pichia pastoris*) when rationally engineered (via computational simulation) by modification of amino acid residues to have a larger binding pocket, exhibited ability to degrade higher molecular weight hydrocarbons compared to non-mutant laccase, even in the absence of mediators (additional cost) [88]. Furthermore, immobilization of laccases on Fe_3O_4 nanospheres coated on silica and chitosan (Fe_3O_4@SiO_2-chitosan) promoted the degradation of anthracene and benzo[a]pyrene by 81% and 69% in 48 h, respectively; and improved operational stability performance [82]. The recovery of these Fe_3O_4 particles containing laccases in their cores, was facilitated through magnetic field; which allowed their reuse, achieving over 50% performance for degradation of these PAHs in three catalytic runs [82]. Additionally, the co-immobilization

of laccase and natural redox mediator (phenolic compounds extracted from soybean meal) on Ca-modified chitosan-alginate support promoted a degradation rate of phenanthrene (94%) about 20-30% higher than that of free laccase and immobilized laccase beads without mediator [90]. Therefore, the aforementioned technologies tend to boost the enzymatic mycoremediation due to these improvements in operational conditions, and possible reductions in process costs [26,73,79].

3. FUNGAL MECHANISMS AND METABOLITES FOR TOXIC METALS REMEDIATION

Bioinoculant-metal interaction triggers enzymatic and non-enzymatic mechanisms related to metal detoxification in fungal cells [91,92]. Fungal exposure to metal promotes oxidative stress that induces ROS formation as well as the elimination of thiols (glutathione and cysteine), important non-enzymatic antioxidants [91]. Antioxidant enzymes such as catalases, glutathione peroxidases and superoxide dismutase are also produced by some strains as a primary defense mechanism [93]. The remediation of heavy metals from fungal bioinoculants can occur by altering metal mobility via immobilization or solubilization mechanisms, or also via enzymatic by reducing metal toxicity due to its transformation from one oxidative or organic complex state to another [7,94].

Fungal bioinoculants can immobilize toxic metals in their biomass by biosorption or bioaccumulation mechanisms, as well as by excretion of metabolites such as chelating agents and pigments [22,30]. Biosorption does not necessarily depend on an active mechanism, i.e. immobilization of the metal can also be achieved by dead fungal cells [7,94]. The potential of fungal bioinoculants as biosorbents of metals emerges from the interaction between metals with chemical groups such as carboxylate, hydroxyl, amino and phosphate of macromolecules (polysaccharides, pigments) that compose their cell wall [25,95]. For instance, chitosan polymer produced from deacetylation of chitin extracted from *Cunninghamella elegans* showed almost similar adsorption capacity for Pb^{2+} and Cu^{2+} (300 $mg \bullet kg^{-1}$) in aqueous solutions or contaminated soils [96]. Likewise, melanin extracted from *Amorphotheca resinae* consisting of indole-based functional groups showed capacity for biosorption of Cu^{2+}, Pb^{2+}, Cd^{2+} and Zn^{2+} in aqueous solutions, as well as constant adsorption/desorption capacity for five cycles [30]. Biosorption mechanisms therefore, can involve ion exchange, adsorption, electrostatic interaction, complexation and precipitation processes [97,98]. In turn, these are highly dependent on parameters such as pH, metal ion concentration and biomass, as biomasses with strong negative charge can adsorb metal ions [99]. Additionally, metal immobilization can also

occur through its biomineralization, when it forms metal-metabolite complexes with precipitating agents produced by fungal bioinoculants, e.g. oxalic acid produced by *Phanerochaete chrysosporium* under metal stress promoted metal detoxification by immobilizing soluble metal ions as metal oxalate crystals [100].

Bioaccumulation process depends on the toxicokinetics and sensitivity of the bioinoculant to absorb the metal in its structure, which may require a longer time for remediation compared to biosorption [94,95]. It is speculated that metal bioaccumulation may occur via a mechanism similar to the influx of metabolically important ions such as Mg^{+2}, K^{+} and Na^{+} into the fungal cells [7]. Some fungi immobilize toxic metals via intracellular peptides such as glutathione, phytochelatin and metallothionein, as these chelating peptides have conserved cysteine residues that allow the formation of metal-thiolate clusters [101]. Bioinoculants composed by AMF, besides the synergistic contribution with plant roots, can carry out bioaccumulation and phytostabilization of metals present in the rhizosphere due to the internalization of metals in their structures (vacuole, cell wall) [36,102]. The main advantage of fungal bioinoculants to accumulate metals is related to their active metabolism that allows their reproductive capacity and modification of the cell surface to absorb them. However, their subsequent recovery is limited due to their internal compartmentalization or precipitation [95,103]. Several mushrooms (edible, inedible and poisonous) are also bioaccumulators of toxic metals, since different metal concentrations can be accumulated along their fruiting bodies (stipe, cap), which depends on the physiology of each species [21,104]. In particular, wild edible mushrooms pose a risk to human health due to their high potential for toxic metal accumulation [104].

Bioinoculants composed of *Glomus versiforme* or *Rhizophagus irregularis* can alter the mobility of metal present in rhizospheres by phytoextraction as they assist metal-plant translocation [36,102]. Metal mobility can also be altered by the action of fungal metabolites such as organic acids, biosurfactants and siderophores (hydroxamates), which promote metal solubilization and sorption/desorption processes [51,92,105]. Although the acid bioleaching by *Aspergillus niger* or *Cladosporium halotolerans* showed promising results for removal of metals such as Pb^{+2}, Zn^{+2} or Mn^{+2} in aqueous solutions, the leaching of sediments can be a source of natural contamination due to the release (solubilization) of metals present in its composition [92,106]. Siderophores increase bioavailability of metals in soils as a mechanism to facilitate Fe^{+2} uptake, which also promotes solubilization of toxic metals [105].

Fungal bioinoculants can carry out oxidation/reduction reactions that biotransform toxic metals into their more stable, less toxic and volatile speciation. These reactions involve the linkage of methyl or alkyl groups through enzymatic mechanisms, which promotes biovolatilization of the metal in its surrounding environment [59,95,107]. However, toxic metals can affect biochemical

mechanisms involved in their detoxification, as they can replace appropriate metal cofactors for enzyme catalysis [108]. In general, less focus has been placed on enzymatic mechanisms in toxic metal remediation, since metals are not decomposed into intermediate metabolites such as petroleum hydrocarbons [91,92]. Laccases from *T. versicolor*, using ABTS, promoted the removal of Fe and Al contained in contaminated soils respectively by 90% and 99% within 35 days [75].

4. FUNGAL BIOSURFACTANTS: THE MULTIFUNCTIONAL MOLECULES

Chemical surfactants are used to increase the availability and mobility of hydrocarbons and toxic metals strongly associated with non-polar domains of soil (i.e., organic matter and micropores), and this promotes better conditions for subsequent remediation of these contaminants [39,51]. Although the combination of chemical surfactants with fungal bioinoculants has promoted better performance in mycoremediation, high concentrations of surfactants can decrease the biotic potential of native soil microbiota [50,109]. These chemicals may also concomitantly cause another environmental problem due to their toxicity and retention in the soil matrix [45,51]. Thus, fungal biosurfactants or biosurfactant-producing fungal bioinoculants are ecological alternatives to reduce environmental toxicity, since fungal metabolism is induced to produce such amphiphilic molecules when the strain is exposed to hydrocarbons [110–112]. The main physicochemical properties of biosurfactants for environmental remediation are related to the reduction of surface/interfacial tension and increase of surface area in different fluid phases, which results in better mobility, solubility, emulsification and bioavailability of contaminants [109,113].

Fungal biosurfactants are directly related to the mechanisms for contaminant absorption by bioinoculants. These molecules can favor hydrocarbons uptake even if the fungal hyphae do not directly touch the contaminant. This fact was demonstrated in a microsystem with *Talaromyces helicus* (biosurfactant producer) and benzo[a]pyrene which were introduced in separate compartments, and even so there was PAH uptake by the fungal strain [24]. Biosurfactants interact with the fungal cell surface through its hydrophilic moiety, which exposes its hydrophobic moiety to the outside and increases the hydrophobicity of the cell surface for contaminant permeation [45]. Similarly, fungal exopolysaccharides interconnect individual cells into a complex mass tangle when these are produced by the strain and/or adhered to its cell wall, which facilitates hydrocarbon uptake [114].

The action of biosurfactants as agents for mobilization of hydrocarbons depends on its Critical Micelle Concentration (CMC), since that at concentrations below CMC there is a reduction of the interfacial force between soil and oil, facilitating diffusion [113]. On the other hand, at concentrations above CMC, aggregated structures are formed as micelles due to its self-assembly property [54,115]. In this context, micelles promote solubilization of hydrocarbons (incorporation), while hydrophilic groups are directed to the surface, i.e., the hydrophobic contaminant is coated by a hydrophilic surface [54,115]. However, higher efficiency process is achieved at higher concentrations compared to CMC, since at lower concentrations surfactants may be degraded or lost on adsorption to the soil [50].

The interaction performance between fungal biosurfactants and toxic metals depends on the chemical structure and ionic charge of the molecule. Biosurfactants can have heteroatoms and/or functional groups (such as carboxyl, hydroxyl and amino) in their structure that by Van der Waals electrostatic interaction form metal-biosurfactant complexes with toxic metal ions [51,116]. Biosurfactants self-assembled as micelles can also interact with oppositely charged metals, which increases the solubility and mobility of the metal and hence its availability [113,117]. Anionic biosurfactants have a strong chelating action for cationic metal ions, while cationic biosurfactants for anionic metal ions [51]. Some cationic biosurfactants are toxic to microorganisms and inhibitory to the biodegradation process even at low concentrations [118]. Non-ionic surfactants exhibit lower CMC values and higher stability in the presence of electrolytes and divalent cations than ionic surfactants, which may require lower surfactant concentrations in the process. However, high adsorption of non-ionic surfactants on soil particles may result in their lower availability [50].

Fungal biosurfactants are molecules with versatile chemical structures, which influence their molecular weight and physicochemical properties. These molecules are composed of polysaccharides, lipids and/or proteins conjugates [45]. Sophorolipids (SL), mannosylerythritol lipids (MEL), polyol lipids (PL), hydrophobins and glomalin are the classes of fungal biosurfactant reported with potential for hydrocarbons or toxic metals remediation [45,119,120]. However, SL have attracted more attention for such purpose probably due to the fact that they are more commercially available [47,48]. In sum, fungal biosurfactants present functional stability even in adverse conditions (temperature, pH, salinity), i.e., they maximize the possible places to be applied and maintain surfactant action even at suboptimal conditions [28,51].

According to the literature, biosurfactants should be able to reduce water surface tension from 72 $mN \bullet m^{-1}$ to values lower than 35 $mN \bullet m^{-1}$ [45]; surfactin (bacterial surfactant) presents values close to 28 $mN \bullet m^{-1}$ [121]. SL, MEL and PL produced respectively by *Meyerozyma* sp., *Ceriporia lacerate* and *Rhodotorula paludigena*, showed surface tension reduction between 31-

33 $mN•m^{-1}$, and emulsification index for hydrocarbons between 77-84%; which corroborates the potential of these biosurfactants for environmental remediation [122–124]. Hydrophobins and glomalin are hydrophobic proteins with surfactant and chelating action [120,125]. Hydrophobin production has been speculated to alter the cellular hydrophobicity of *Aspergillus brasiliensis* during hexadecane uptake [32]. Overexpression of genes encoding hydrophobins during cultivation of *Trichoderma harzianum* in mercury-containing medium has also been reported to facilitate bioaccumulation of the metal [120]. Glomalin is produced by AMF, and acts in the maintenance of soil properties (particle aggregation and stability) [119]. This protein showed ability to immobilize Zn^{+2} and Pb^{+2} in aqueous solutions [126], and to reduce phenanthrene adsorption in soil (increasing bioavailability for remediation) [125].

4.1 Adjuvants for biostimulation and bioaugmentation processes

Fungal biosurfactants are adjuvants that can enhance biostimulation and/or bioaugmentation processes. A schematic chart on strategies for the use of fungal biosurfactants in remediation of soils contaminated with petroleum hydrocarbons is presented in Figure 5. The addition of biosurfactants increases the availability of the contaminant, which promotes its subsequent assimilation/degradation by the autochthonous microbial community (biostimulation) (Fig. 5-a) [109,127]. Fungal biosurfactants can also be assimilated as substrates for autochthonous microbiota [47]. On the other hand, the addition of biosurfactant-producing fungal bioinoculants compatible with native soil microbial communities can maximize remediation efficiency (bioaugmentation) (Fig. 5-b). Combining the use of biosurfactants and bioinoculants can minimize the microbial load (inoculum size) required in the mycoremediation process and achieve similar results [109,118]. Furthermore, it is assumed that fungal biosurfactants in biostimulation/bioaugmentation processes increase bioavailability of substrates due to solubilization of organic matter, which can stimulate fungal growth. However, surfactant concentration may also influence the activity of enzymes involved in the process [50].

Fig. 5. Fungal biosurfactants can be added with micro- and macro nutrients for biostimulation (a) of autochthonous fungi involved in remediation, while the addition of fungal bioinoculants (autochthonous or allochthonous) that produce biosurfactant can increase efficiency due to microbial bioaugmentation (b). Fungal biosurfactants can also be added to washing solutions to facilitate contaminant desorption/solubilization, and achieve higher removal rates (c)

Mannoproteins produced by *Saccharomyces cerevisiae* promoted a two-fold higher degradation of soy biodiesel when added to unsterilized contaminated soils compared to sterilized soils, i.e., the biosurfactant potentiated the biostimulation process [128]. The use of biosurfactants produced by *Candida sphaerica* [39] and *Starmerella bombicola* [23] combined to processes of biostimulation with sugarcane molasses and bioaugmentation with their respective producing

yeast, besides reducing the time required to reach the same level of degradation in these processes without biosurfactant, also promoted the degradation of 50% and 88% of motor oil in sand, respectively [23,39]. Moreover, bioaugmentation processes applied to soils contaminated with biodiesel (20%) by inoculation (10%) of *A. niger* in solid fermented medium (containing biosurfactants and lipases) promoted a 10% higher degradation compared with natural attenuation process within 60 days duration [129].

4.2 Adjuvants for washing processes

Soil washing processes containing surfactants even at concentrations below their CMC increases the solubility of hydrocarbons and toxic metals (Fig. 5-c) [130,131]. Application of surfactant-containing wash solutions can be carried out by techniques including flooding, basin infiltration system, infiltration well, and leach field [50]. Biosurfactants increase the transfer of the contaminant to the aqueous phase. They reduce the adhesion between the contaminant and the soil matrix, as well as contaminant viscosity [28,109]. SL, when added to washing solutions, increase the capacity for Fe, Cu and As removal compared to water alone [132], as well as show superior potential to synthetic surfactants (sodium dodecyl sulfate and Tween 80) for Cd and Pb removal during soil washing [133].

Biosurfactants from *C. sphaerica*, *C. tropicalis* and *Rhizopus arrhizus*, when applied to artificially contaminated sand, were effective in removing adsorbed engine oil (65%), petroleum (78%) and diesel oil (79.45%), respectively [134–136]. Likewise, SL produced by *S. bombicola* showed superior results (68%) when compared to the chemical surfactant Triton-X (38%) for the removal of kerosene (C_{10} - C_{40}) from a contaminated soil [130]. The performance of washing soils with SL in packed columns depends on the granulometry, porosity and permeability of soils, since the removal of motor oil in sandy soil and beach sand was approximately 3 and 4-fold higher than in silt and clay soil, respectively, due to a better mass transfer during percolation of the washing solution [23].

Toxic metal removal from soils by washing can be enhanced with prior speciation of components, shapes or phases, in which the metallic elements occur [137]. Metals in exchangeable hydroxides state, carbonates and reducible oxides are more easily removed, unlike residual fractions, during the washing process [117]. Soil washing processes do not necessarily require highly purified biosurfactants, those produced by a *Y. lipolytica* removed 30-40% Cu and Pb in artificially contaminated sand, while the same purified ones also removed approximately 30% [116].

The washing of soils contaminated with toxic metals can have its performance influenced by the chemical composition and concentration of biosurfactant, temperature [132,133] and the use of other adjuncts such as acids and bases [131]. Acidic SL produced by *S. bombicola* at 8% concentration when added to washing solutions promoted removal of Cd (83%) and Pb (45%) in artificially contaminated soils. However, due to their lower water solubility, lactonic SL at 1% concentration promoted lower removal of Cd (10%) and Pb (4%) in these soils [133]. Biosurfactants from *C. sphaerica* were superior to chemicals used in acid and basic leaching for the removal of Pb (70%), Fe (89%) and Zn (87%) from contaminated soils, even at concentrations lower than the CMC [131]. The temperature of the wash solution containing acidic and lactonic SL when increased from 15 to 23°C also increased the removal of arsenic by 11%, but this potential was decreased by 22% when the temperature was increased from 23 to 35 °C, probably due to changes in properties involving metal-biosurfactant interaction [132].

5 SELECTION OF FUNGAL BIOINOCULANTS AND/OR METABOLITES FOR REMEDIATION

The convergence between the premises that nature still has under-exploited resources and fungi have a ubiquitous habitat is the driving force for bioprocesses development aimed at exploiting biodiversity through bioprospecting technologies, which involves the isolation and screening of potential bioinoculants and/or metabolites for environmental remediation. The isolation of fungal strains can promote the discovery of new species or species not yet described for mycoremediation, as well as promote the domestication of these isolates for use in bioprocesses related to the production/application of bioinoculants and metabolites for environmental remediation [138,139]. Although extreme environments such as cold [140], saline and marine (high pressure and depth) [31,141] have been resource to isolate potential fungal bioinoculants and metabolites for remediation of hydrocarbons and toxic metals, these environments are under explored compared to contaminated soils and mine tailings [17,92].

It is not a fallacy to report that technologies related to bioprospecting and screening of bioinoculants, and metabolites are exhaustive and tiresome similar to the route of a treasure map, but these technologies give accessibility to find possible fungal treasures to be used in bioprocesses focused on environmental remediation. Thus, the development of methodologies to reach such treasures is based on consolidated scientific methods and on the current scientific and technological progress to provide technologies related to the bioprospecting of fungal

strains in order to effectively isolate and screen a larger number of strains and present them in environmental remediation strategies.

5.1 **Isolation, the first step towards unveiling hidden potential**

Although contaminated environments can promote a toxic effect on the local microbiota, they can also promote an enriching effect to isolate more resistant autochthonous fungi than allochthonous ones to be used in remediation of hydrocarbons and toxic metals [142–144]. Fungi thrive in contaminated environments as their metabolic pathways are modulated to respond to both the nutrient and the stress state (e.g., presence of contaminants) in order to efficiently allocate energy from constitutive to conditional expression [68]. Thus, enrichment of soils (contaminated or not) with hydrocarbons or toxic metals; and/or with micro and macronutrients for a period of time before isolation may promote greater chances of selecting potential fungi able to assimilate/interact with contaminants or produce metabolites [17,144]. This statement is true considering that fungal metabolism is induced to produce enzymes and/or biosurfactants under such conditions for the strain to thrive in the nutrient medium (with or without a specific contaminant) [110,111]. The fungal biodiversity, when explored through cultivation-dependent techniques, results in a practically "negligible" number of recovered individuals in comparison to the real "hidden community" that thrives there, since some microbial strains may be neglected because they are not cultivable under laboratory conditions and/or require selective technologies to promote appropriate conditions for their isolation [145,146]. These selective technologies can be based on aspects related to cellular composition (cellular hydrophobicity) or microbial metabolism (growth rate) to promote a targeted isolation of fungal strains that potentially interact with contaminants or that would be presumably hidden [146,147]. For example, the mineral oil flotation technique may preferentially select the isolation of fungal strains with a hydrophobic surface (assimilate hydrocarbons by passive mechanism), as these strains tend to concentrate at the oil/salt solution interface due to the hydrophobicity of their cell wall [147]. Moreover, microbial exposure to the atmosphere of aromatic compounds selects fungi that are resistant to the toxic gases, as well as prevents possible slow-growing, low-competitive bioinoculants from being overlooked due to the rapid growth of other fungal strains during isolation [146].

5.2 Screening/Bioprospecting

After isolation, the selection of possible bioinoculants and/or metabolites are screened from technologies that may involve colorimetric, gravimetric, respirometric, chromatographic, spectroscopic, enzymatic and/or tensiometric analysis [44,56,139]. However, screening a high

number of fungal isolates requires simple, fast, inexpensive, and most importantly accurate and reproducible techniques for all of them [148,149]. The main qualitative and quantitative methods for screening potential fungi to be applied in hydrocarbon remediation are based on the growth of strains in specific media, both solid and liquid, to analyze their ability to assimilate the contaminant and/or ability to produce enzymes or biosurfactants [41,43,111]. The combination of screening techniques provides a more accurate answer on the selection of fungi for bioprocesses focused on environmental remediation, since each individual technique has different sensitivity and coverage, which may overlook some potential strains if not selected by a specific method [44,144,147]. For example, solid media are more susceptible to false-positive results for metabolite screening and may limit mass transfer of metabolite in the media [45].

In this context, solid media containing hydrocarbons are commonly used for an initial screening of strains able to resist or tolerate contaminants [17,150]. Colorimetric tests such as phenolic oxidation (gallic acid, tannic acid, guaiacol) and Remazol Brilliant Blue R (RBBR) decolorization are used to qualitatively screen fungi that degrade hydrocarbons [149]. The phenolic oxidation test analyses the excretion of phenol oxidases, which results in the formation of a brown halo around the mycelium [44,149]. The ability of the fungus to decolorize RBBR is due to its metabolism in excreting enzymes (principally laccases) involved in the degradation of aromatic contaminants, which promotes decolorization from blue to yellowish white [44]. Regarding the bioprospecting of biosurfactant-producing fungi, the screening can be carried out by colorimetric methods such as blood hemolysis and methylene blue - cetyltrimethylammonium bromide (CTAB) that involves the excretion of this metabolite in solid culture media [45].

In liquid culture media, screening of strains for degradation/assimilation of hydrocarbons commonly occurs by simple tests such as gravimetric analysis of the degraded contaminant [17], profile for enzyme and biosurfactant production, and biomass dry weight [17,56]. The 2,6-dichlorophenol indophenol (DCPIP) is an indicator (redox dye) that analyses excretion of oxide-reductases by fungal strains in the presence of hydrocarbons [43,151]. DCPIP in its oxidized form shows a blue color, which becomes colorless when this indicator is reduced by oxido-reductases during hydrocarbon biodegradation [17,43,56]. Tensiometric analysis and/or emulsification index of liquid culture supernatants of fungal strains provides more accurate results than colorimetric analysis for screening fungi producing biosurfactants, as these technologies allow the measurement of surface tension and/or ability to emulsify hydrocarbons [45,111,139].

The measurement of the fungal dry biomass weight after its growth in liquid medium containing hydrocarbons allows estimating which strains can better assimilate or degrade contaminants, as

well as which chemical structure profiles the strain has more capacity to degrade [17,138]. It is roughly believed that fungal dry biomass weight reveals the ability of the fungus to convert hydrocarbon into energy to produce biomass, even if not all carbon source was targeted for such purpose [151,152]. This dry biomass method is analogous to the gravimetric method for screening fungal bioinoculants that assimilate hydrocarbons in liquid media. However, the difference between these technologies is related to the biomass or contaminant that will be measured in the final screening step [33,141]. Additionally, fungal biomass can have the hydrophobicity of its cell surface measured to correlate with its potential to produce biosurfactants and absorb hydrocarbons [110,153].

Other quantitative methods, such as gas chromatography (GC) and respirometry, are less used to screen fungal isolates as possible bioinoculants for hydrocarbon remediation [56,61]. Although GC provides accurate information on the conformational changes in hydrocarbon structure after mycoremediation, this technology is still expensive for screening processes [61]. The respirometry technique can screen fungi by quantifying CO_2 produced by the strain during mycoremediation, its main product generated when hydrocarbons are mineralized by aerobic degradation [56,154].

The screening of possible fungal bioinoculants for the remediation of toxic metals also occurs from the growth of strains in liquid or solid culture media containing toxic metals to initially measure the tolerance of strains to metals through analysis of minimum inhibitory concentration (MIC) [55,155]. However, only more robust and sophisticated techniques such as atomic absorption spectrometry (AAS) can quantify metal removal, as well as elucidate the type of strain-metal interaction [25,144].

6 MOLECULAR APPROACHES: THE KEY TO UNRAVELLING ANSWERS AND EXTRAPOLATING RESULTS

It is conceivable that fungal strains have evolved in the direction of ecological fitness rather than biotechnological efficacy, i.e., bioengineering of strains is a prerequisite for increasing the metabolic efficiency in mycoremediation [58]. DNA decoding of fungal strains and other molecular approaches will certainly be needed in every mycoremediation process that involves bioprospecting or attempts to propose degradation steps, elucidate regulatory mechanisms, monitor microbial profile, enhance strains for productivity increases, etc. There was a gap of 20 years between the use of fungal bioinoculants to the proper use of their metabolites for mycoremediation, as more attention was paid to this purpose after the sudden accelerated expansion of molecular techniques. Currently, -omics technologies are technological resources

for expanding molecular approaches in the development of bioinoculants and metabolites for remediation of hydrocarbons and toxic metals, as well as for understanding microbial interaction on contaminated sites or during remediation.

6.1. Molecular characterization and microbial monitoring techniques

Fungal isolates with potential to be used in mycoremediation can also be potentially opportunistic pathogens or be closely related at molecular level to plant, animal and/or human pathogens; e.g., *Fusarium oxysporum* and *Paecilomyces variotii*. This fact promotes biological risk due to virulence factors and spore respiration in bioaerosols [4,141]. Any fungal bioprocess must meet biosafety criteria. Thus, morphological and molecular identification of the isolate is essential to previously verify its pathogenic potential before proceeding with its use in mycoremediation [4,156]. However, a single fungus isolated from a contaminated environment can show large morphological differences, since these variations can be due to mutations caused by the high concentration of contaminants and broad resistance/tolerance mechanisms that apply to each isolate [157,158]. Therefore, the fungal strain should preferably be identified from its gene sequence through the Internal Transcript Spacer (ITS) region and, if necessary, other genes could be used, such as beta tubulin, actin, elongation factor [6,33]. The results are compared with similar type strains of different species within the genus through multiple sequence alignment, ensuring correct taxonomic identification [53,58].

Several PCR-based fingerprinting genotyping techniques are available to profile fungal isolates, as well as to monitor diversity and abundance of microbial communities and bioinoculant survival during mycoremediation [159,160]. In synthesis, these technologies are related to denaturing gradient gel electrophoresis (DGGE) [41,160], fluorescence *in situ* hybridization (FISH) [161] and terminal restriction fragment length polymorphism (T-RFLP) analysis [159]. Quantitative PCR (qPCR) has been used to quantify bacterial (16S) and fungal (ITS) genes as indicative of microbial abundance, monitor catabolic activity and gene expression during mycoremediation [6,40,44]. Moreover, DNA microarray technique has identified genes regulated in response to exposure to high concentrations of toxic metals [162].

6.2 Genetic engineering and heterologous production

Advances in technologies related to fungal genome sequencing have been a shortcut to achieve improvements in bioprospecting for potential bioinoculants, enzymes and biosurfactants through data mining as well as to infer functions of genes involved in hydrocarbon degradation [45,161,163]. For example, the screening of bioinoculants that degrade aromatic hydrocarbons can be carried out from the search of sequences encoding the *C23O* (catechol 2,3-dioxygenase) gene [161]. Furthermore, silencing of the *CYP52L1* gene (CYPs family) by post-transcriptional

gene silencing (RNAi) in *Graphium* sp. showed that the reduced monooxygenase activity, as well as the ability of this fungus to grow on alkanes and ethers was extinguished [163].

The elucidation of catabolic genes related to hydrocarbon or heavy metal remediation allows designing fungal strains of higher performance due to the possibility of recombination/expression of these genes in another/single host [26,160,164]. Although post-translational modifications during the processing of phenol oxidases are more effectively carried out by native lignolytic fungi than in host microorganisms, several host fungi have shown promising results in heterologous production of these enzymes for mycoremediation [89,165]. Genes encoding for laccases from the basidiomycete *Trametes* sp. were expressed in ascomycete hosts such as *A. niger* [166], *Trichoderma atroviride* [167], *Y. lipolytica*, *S. cerevisiae* and *P. pastoris* [165]. The bioinoculant *A. niger* genetically engineered for expression of genes encoding lignin peroxidase and manganese peroxidase from *P. chrysosporium* enhanced the degradation of phenanthrene, pyrene and benzo[a]pyrene in soil microcosms [160]. The yield of recombinant thermophilic tyrosinase from *Thermothelomyces thermophila* was increased 2-fold when this enzyme was cloned and expressed in *P. pastoris* rather than in other microbial cells [168].

Insertion and/or deletion of some genes of specific interest in host strains enhance mycormediation processes. For example, increasing the active site of the non-lignolytic enzyme CYP63A2, among other CYP family enzymes, was achieved by expressing CYPs together with cytochrome P450 reductases from *P. chrysosporium* into *P. pastoris*, which promoted the oxidation of different contaminants such as PAHs, alkylphenols and long-chain alkanes [169]. Similarly, the insertion of the lipase gene *PaLIPAp* from *Pseudozyma antarctica* T-34 into *Pseudozyma tsukubaensi* increased the uptake of oily contaminants, besides promoting a 1.7-fold yield of MEL production [26], a biosurfactant that has already been regarded as promising for mycoremediation [45]. Furthermore, the insertion of the metallothionein gene *PtMT2b* from *Populus trichocarpa* into *S. cerevisiae* increased approximately 10-fold the tolerance and bioaccumulation of Cd when compared to its wild counterparts [164]. Additionally, the deletion of the *crpA* gene (P-type ATPase) in *Aspegillus nidulans* increased Cd biosorption capacity by 2.7-fold [170].

6.3 The “-omics” technology

Several complete or near complete genomic sequences of culturable microorganisms with potential in mycoremediation are available in online databases, as well as sequences of catabolic genes also involved in such processes [1,58]. However, it is estimated that a 99% gene pool of microbial diversity remains unknown due to limitations of culture-dependent techniques [145,171].

In this context, genomic approaches of fungal isolates allow access to the possible metabolic pathways related to enzyme and biosurfactant production [31,45]. For instance, the whole genomic analysis of *A. sydowii* allowed deducing possible enzymatic pathways of hydrocarbon degradation when combined with GC-MS, and revealed a variety of genes involved in the biodegradation of aromatics, such as monooxygenases, dioxygenases, glutathione transport system, peroxidases and semialdehyde dehydrogenase [31].

On the other hand, metagenomic approaches allow elucidating the microbial interaction at the contaminated site or during mycoremediation without the need for microbial cultivation [58,145]. Metagenomic analysis of total DNA from contaminated environmental samples allows the screening of metabolite-producing fungi applied to remediation through genomic data mining or functional screening of specific genes [172,173]. However, metagenomic studies on fungi remain sparsely explored, and the results of annotated sequences for eukaryotes is much lower than for bacteria [173]. For instance, functional metagenomic analysis of petroleum contaminated soils when bioaugmented with *Ciboria* sp. revealed synergy between fungal bioinoculant and different taxa of hydrocarbonoclastic bacteria (*Streptomyces, Nocardoides, Pseudonocardia, Solirubrobacter, Parvibaculum, Rhodanobacter, Luteiomonas, Planomicrobium* and *Bacillus* spp.) during contaminant degradation [138]. A metagenomics study also revealed that the fungal community possibly involved in PAHs remediation, differed by approximately 30% between soils collected at 0.5-meter (m) distance from oil wells compared to those collected at 3 m [119]. Furthermore, the genera *Tetracladium*, *Exophiala*, *Schizothecium* and *Ilyonectria* identified in samples collected at 0.5 m, showed strong correlation with PAH content, which classifies them as potential bioinoculants for mycoremediation [119].

Transcriptomic (mRNA profile), proteomic (proteins) and metabolomic (metabolites) analyses elucidate changes in the composition, regulation (up-regulated or down-regulated) and abundance of differentially expressed genes, proteins and metabolites under a given environmental/specific condition [53,174,175]. These omics technologies provide important information to maximize performance in using fungal bioproducts to remediate hydrocarbons and toxic metals [53,174,175].

The transcriptional response of *Y. lipolytica* after exposure to uranium (50 μM) revealed the expression of 33 upregulated genes, which are involved in metal transport, DNA repair and oxidative stress response, as well as another 23 downregulated genes, which are involved in cell wall and cell cycle [174]. Through transcriptomic analysis, the differential expression of the chloroperoxidase enzyme-related *cpo* gene, not previously described for PAH degradation, was identified during benzo[a]pyrene degradation under hypersaline conditions by *A. sydowii* [53].

Furthermore, enzymatic mechanisms involving mitochondria membrane-bound enzymes (CYPs, dehydrogenases, quinone reductases) and cytosol-soluble enzymes (dioxygenase, glutathione transferase) for PAHs degradation by *A. sydowii* were proposed through a combination of transcriptomics and metabolomics [53].

Recently, proteomic analysis allowed the elucidation of n-hexadecane transmembrane transport mechanisms in *C. tropicalis*, since the 231 proteins differentially expressed by this strain are clearly enriched in endocytosis and phagosome pathways, as well as probable involvement in the energetic metabolism of the strain [175]. Metabolomics analysis detected intermediate metabolites such as hydroxy naphthoic acid and catechol synthesized from oxidation of phenanthrene and benzo[a]pyrene by *A. sydowii*, as well as allowed the prediction that degradation of these contaminants occurred in approximately 10 days, since no aromatic metabolite was detected in samples taken after this period [53].

7 BIOPROCESS ENGINEERING TO BOOST MYCOREMEDIATION: AN OVERVIEW OF BIOPROCESSING

The use of technologies integrated with engineering principles and appropriate maintenance of requirements related to bioprocessing involved in production or application of bioproducts will probably promote more and more possibilities for consolidation of mycoremediation. Bioprocess engineering and its technologies investigate and/or enhance the performance of steps related to the development of bioinoculants and fungal metabolites in order to ensure efficiency in production, application and ecotoxicological safety [41,176].

Fungal bioprocesses for the production or application of bioinoculants and metabolites for remediation of hydrocarbons and toxic metals present an attribute of alterity. Thus, although there are intrinsic particularities related to the production process, as type of cultivation (submerged, semi-solid or solid), or issues related to the application, as type of site (soil, sludge, or water), these bioprocesses also present similarities related to subjects like the influence of nutrition and environmental parameters, complexity of each step along the process, physiological state, and metabolism of the inoculum [45,46]. Alternatively, experimental designs combined with statistical tools such as response surface methodology has allowed to select/evaluate optimal operating conditions (e.g. inoculum size, pH, etc.) and interaction between nutritional and environmental parameters to enhance the performance of fungal bioinoculants and/or metabolite production for mycoremediation [144,177]. However, although technologies related to batch, fed-batch and semi-continuous cultivation systems promote yield or efficiency enhancements in bioinoculant or metabolite production, the use of these

technologies for application in mycoremediation is still under-explored probably due to practical limitations [75].

In this topic, technologies developed for production and application of bioinoculants, enzymes and biosurfactants for remediation of hydrocarbons, and for toxic metals will be approached. Those related to bioprocessing for production of enzymes and biosurfactants will be presented only when these metabolites were produced exclusively for remediation purposes.

If the reader is interested in the large-scale bioprocessing of fungal surfactants and enzymes, these have been described in detail by da Silva [45] and Fasim [46], respectively.

7.1 Inoculum as bioinoculant product

The inoculum is the active portion of the microorganism used in remediation processes. When the inoculum is the bioproduct to be used, this is termed bioinoculant. Thus, the inoculum is the main promoting agent or product for mycoremediation, therefore its efficiency is essential to achieve better performances [138,178]. On a laboratory scale, mycoremediation requires the use of a standardized inoculum, especially when the aim is to evaluate efficiency after remediation. The initial steps involved in fungal inoculum preparation include strain growth in solid medium and its sequential growth in liquid medium [28,179]. After recovery of fungal biomass (inoculum), washing steps with water [180], saline solution [138] and buffer solution [181] can be carried out to standardize the inoculum.

As a rule of thumb, filamentous bioinoculants are generally used at a cell concentration ranging from 10^4 to 10^8 spores • [mL or g]$^{-1}$ [179,182]. Yeast bioinoculants are quantified indirectly by optical density of the culture medium, and an absorbance between 0.6 and 1.0 (at 600 nm) is indicated for better process performance [19,128]. Modulation of inoculum size for bioinoculant application or metabolite production ranges from 5 to 10% of the total process volume [27,28]. A 7- and 5-fold increase in the inoculum size of *Ciboria* sp. and *Mucor hiemalis* increased oil degradation in soils by 27% [138] and biosurfactant production by 560% [28], respectively. In general, a practical application of fungal bioaugmentation will certainly require a greater inoculum amount than under controlled conditions, which would aim to minimize effects of microbial competition and ensure their development under adverse conditions [60,118].

In this context, alternative technologies for preparation/use of bioinoculants have been developed in uncontrolled conditions that resemble the real environment of an application, and/or in conditions that can induce the fungal metabolism to increase its performance before the beginning of mycoremediation [32,178]. For example, the bioinoculant *Pleurotus ostreatus*,

when previously submitted to an acclimation treatment in lignocellulosic biomass, promoted greater efficiency in diesel degradation after its inoculation in soil [178]. Regarding metabolites production, the application of an electric field to the developing inoculum (spores) of *A. brasiliensis* increased the bioemulsifier production (19.5%) applied to PAH and aliphatic remediation [32].

Morphological structures of fungal bioinoculants directly influence mycoremediation. Various fungal structures such as spores, mycelia, pellets and/or fruiting bodies of mushrooms were analyzed for remediation of PAHs and metals in soils [21,183]. Mycelial inoculation promoted higher degradation rates of PAH compared to the use of spores of *Coniothyrium* sp. (26.5%) and *Fusarium* sp. (27.5%) [182]. The use of mycelium from *Bjerkandera adusta* increased PAH degradation by up to 29% when compared to the use of pellets, probably due to higher oxygen transfer [183]. Similarly, the inoculum of *Aspergillus ochraceus* with concentration at 1% ($m \bullet v^{-1}$) in its fresh mycelium state ($v \bullet v^{-1}$) showed higher performance (13%) compared to its spores at 5% ($v \bullet v^{-1}$) for oil degradation in liquid culture [184]. Fungal bioinoculants of *Galerina vittiformis* showed approximately 4-fold higher bioaccumulation capacity of Cd^{+2}, Cu^{+2}, Cr^{+6}, Pb^{+2} and Zn^{+2} in its fruiting body state than in its mycelium, i.e. fungi that form fruiting bodies may also have an advantage as they are easily separated from soil [21].

There are implications related to fungal morphology in bioprocesses for environmental remediation, which directly influences the process and can presume its best morphological structure for specific application conditions [21,184]. In this sense, dimorphic fungi (e.g., black fungi) in their filamentous structure may be more viable to be applied as bioinoculants, because hyphae can penetrate into the contaminated soil matrix pores, which allows greater accessibility and permeation to contaminants between different regions of the mycelium and through the air [101,182]. On the other hand, dimorphic fungi in their yeast-like form may be more viable to be applied in bioprocesses as producers of metabolites to be applied in mycoremediation, because the yeast morphology facilitates biomass recovery [185].

Although bioinoculants of different phyla and order have shown high efficiency for mycoremediation of hydrocarbons and toxic metals due to their biochemical mechanisms or metabolites produced, the main process conditions (e.g., contaminant type, bioprocess type) and parameters (e.g., time, pH, temperature, agitation) under which each experiment was carried out should be individually considered in detail to conclude on this efficiency more accurately. In this context, table 1 presents data on a diversity of possible fungal bioinoculants, process condition and parameters involved in hydrocarbon remediation. Similarly, table 2 presents data on toxic metal remediation.

Table 1 – An overview of hydrocarbon remediation processes by bioinoculants and metabolites

Phylum/Order	Strain	Contaminant	Metabolite	Removal efficiency (%)	pH/ (%) moisture	Tempera-ture (°C)	Mixing (rpm)	Time (days)	Data source	Reference
Mucoromycotina										
Mucorales	*Mucor racemosus*	ANT	ND	12-14	ND	32	130	14	LC	[38]
Ascomycota										
Hypocreales	*F. solani*	BAP	-	16	ND/25	-	-	9	SM	[6]
	Fusarium neocosmos-poriellum	crude oil	LCC/BS	43	ND/60	-	-	150	SM	[186]
	Purpureocillium lilacinum	crude oil	-	44	7/100	30	-	40	LC	[17]
	Acremonium sclerotigenum	n-alkanes	-	52	-	28	80	23	LC	[33]
	Trichoderma tomentosum	PHE, ANT, PYR	-	60-90		25	100	49	LC	[56]
Pleosporales	*Alternaria alternata*	diesel oil	LCC, MnP	28	-	26	-	28	LC	[152]
	Coniothyrium sp.	PHE, ANT, PYR	-	26	-	-	-	30	LC	[182]
	Ulocladium sp.	PHE, BAP, PYR	LCC, MnP	40-98	ND/20	-	-	120	SM	[41]
Eurotiales	*Penicillium citrinum*	n-alkanes	-	80	-	28	80	23	LC	[33]
	Aspergillus ustus	crude oil	-	30	7/100	30	-	40	LC	[17]
	A. niger	biodiesel	lipase/BS	64	ND/60	-	-	60	SM	[129]
	P. variotii	diesel oil	MnP, CAT	30	-	26	-	28	LC	[152]
	T. helicus	BAP	-	33	-	-	-	9	SM	[6]
Saccharomycetales	*C. maltosa*	alicyclic hydrocarbons	-	30-38	-	30	300	5	LC	[62]
	Lipomyces tetrasporus	crude oil	-	68		28	150	30	LC	[141]
	C. tropicalis	saturated and aromatic	DH, PPO, BS	42-96	2/60	-	-	180	SM	[181]

		hydrocarbons								
	Yamadazyma mexicana	octane, PYR	-	24	-	30	150	360	LC	[187]
Capnodiales	*Cladosporium* sp.	PYR, PHE, ANT, FLU	ND	47-71	-	32	130	21	LC	[38]
	Cladosporium sphaeros-permum	diesel oil	LCC, LiP, CAT	40	-	26	-	28	LC	[152]
Microascales	*Pseudoallescheria* sp.	aliphatic hydrocarbons	LCC/hydrolases	79	ND/50	28	-	60	SM	[180]
Helotiales	*Cadophora* sp.	PHE, BAP	LCC, MnP	74-99	-	25	150	10	LC	[188]
incertae sedis	*Trematophoma* sp.	PHE, ANT, PYR	LCC	56-90	-	28	180	15	LC	[189]
	Pseudogymnoascus sp.	PHE, BAP	LCC, MnP	53-93	-	25	150	10	LC	[188]
Basidiomycota										
Agaricales	*Pleurotus dryinus*	PHE, FLU, PYR	LCC	25-60	7	27	150	30	LC	[179]
	P. ostreatus	PHE, BAP	LCC, MnP	64-98	-	30	150	10	LC	[188]
	Pleurotus florida	saturated and aromatic hydrocarbons	LCC, tyrosinase, BS	55	-	24	120	30	LC	[71]
	Crucibulum laeve	PHE, PYR, BAP	DH, LCC, PPO	42-64	ND/60	20-28	-		SM	[190]
Polyporales	*Trametes polyzona*	PHE	LCC	98	4.5/100	-	150	1	LC	[35]
	P. sajor-caju	phenol	LCC	36-82	6.5/100	28	-	4	LC	[27]
	Megasporoporia sp.	BAP	LCC, MnP	54	-	30	150	14	LC	[43]
	Microporus vernicipes	PHE, ANT, FLU, PYR	LCC, MnP, LiP	15-38	-	27	150	14	LC	[44]
	Phlebia acerina	PHE, ANT, FLU, PYR	LCC, MnP, LiP	42-52	-	27	150	14	LC	[44]
	Trametes hirsuta	phenol, PHE, ANT, FLU, PYR	LCC	30-85	7	27	150	30	LC	[179]
	Polyporus sp.	CHRY	1,2-CTD	65	-	25	120	30	LC	[67]
Sporidiobolales	*Rhodotorula ingeniosa*	octane, PYR	-	35	-	30	150	360	LC	[187]

Russulales	*Peniophora incarnata*	PHE, ANT, FLU, PYR	LCC, MnP, LiP	68-95	-	27	150	14	SM	[44]

Caption - ND: not described, BAP: benzo[a]pyrene; PHE: phenanthrene; ANT: anthracene; PYR: pyrene; FLU: fluoranthene; CHRY: chrysene BS: biosurfactant; LCC: laccase; MnP: manganese peroxidase; CAT: catalase; LiP: lignin peroxidase; DH: dehydrogenase , PPO: polyphenoloxidase; 1,2-CTD: Catechol 1,2-dioxygenase; SM: soil microcosms; LC: liquid culture

Table 2 – An overview of toxic metals remediation processes by bioinoculants and metabolites

Phylum/Order	Strain	Metal	Mechanisms	Removal efficiency (%)	pH/(%) moisture	Tempera-ture (°C)	Mixing (rpm)	Time (h)	Data source	Reference
Glomeromycota										
Glomerales	*G. versiforme*	Cd	Phytoextraction	25-74	ND/60	22-28	ND	1680	SM	[36]
	R. irregularis	Cd	Phytoextraction	ND	ND	22-28	ND	1680	SM	[102]
Mucoromycotina										
Mucorales	*Circinella* sp.	Ni	Biosorption	6	6/ND	40	ND	1	LC	[191]
	Cunninghamella sp.	Pb	Biosorption	95	ND	28	180	168	LC	[192]
	Mucor circinelloides	Pb, Cd, As	Biosorption/Bio-accumulation	2-87	6/ND	25	170	35	LC	[42]
	Rhizopus stolonifer	Pb, Cd, Ni	Bioaccumulation	17-59	ND	30	150	96	LC	[18]
Ascomycota										
Coniochaetales	*Lecythophora* sp.	Hg	Biovolatilization/ Bioaccumulation	86	ND	30	150	96	LC	[143]
	Lecythophora sp.	Hg	Bioaccumulation	13-26	ND/50			1344	SM	[143]
Eurotiales	*A. niger, A. fumigatus, A. flavus*	Hg, Pb	Bioaccumulation	96	ND	30	120	120	LC	[155]
	Aspergillus lentulus, A. terreus	Cu, Cr	Biosorption	65-95	ND	30	150	120	LC	[55]
	Paecilomyces sp.	Cd	Bioaccumulation	35	6/ND	28	120	240	LC	[157]
	Talaromyces islandicus	Pb	ND	80	ND	30	ND	120	LC	[193]
Hypocreales	*Trichoderma lixii*	Cu	Biosorption	25-85	7/ND	28	80	120	LC	[34]
	Beauveria bassiana	Pb	Biomineralization	8	7/ND	30	ND	504	LC	[194]

Microascales	*Scopulariopsis brevicaulis*	Hg, Se	Bioaccumulation /Biovolatilization	46-50	5,6/ND	25	ND	720	LC	[107]
Saccharomycetales	*S. cerevisiae*	U (VI)	Biosorption	75	3/ND	30	150	24	LC	[20]
Pleosporales	*Microsphaeropsis* sp.	Cd	Biosorption	90	6.5/ND	30	ND	72	LC	[25]
Basidiomycota										
Polyporales	*P. chrysosporium*	Cd	Biosorption	47	ND	30	120	432	LC	[100]
	T. hirsuta	As, Cu, Fe, Cd, Zn, Pb	Biosorption	23-98	7/ND	27	150	720	LC	[179]
Agaricales	*P. ostreatus*	Cd	Biosorption	54	6/ND	30	100	168	LC	[195]
	Schizophyllum commune, Leucoagaricus naucinus	U (VI)	Biosorption/Bioaccumulation	10-60	ND	ND	120	168	LC	[22]
	G. vittiformis	Cd, Cu, Cr, Pb, Zn	Bioaccumulation	10-90	ND/85	22	ND	600	SM	[21]
Tremellales	*Cryptococcus laurentii*	Al, Cr, In, Ga, Fe, Bi	Biosorption	60-80	4-7/ND	ND	500	1,5	LC	[185]
	Trichosporon sp.	Pb, Zn, Ag	Biosorption	ND	ND	28	ND	48	LC	[103]
Sporidiobolales	*Rhodotorula mucilaginosa*	Zn, Pb	Biomineralization	2-16	7/ND	30	ND	504	LC	[194]

Caption: - ND: not described, SM: soil microcosms; LC: liquid culture

According to Tables 1 and 2; bioinoculants belonging to the phyla Ascomycota and Basidiomycota are the most explored for mycoremediation of hydrocarbons and toxic metals, while the phyla Glomeromycota and Mucoromycotina have been more applied to remediate metals. Trials with bioinoculants for mycoremediation of metals are generally carried out in shorter time when compared to hydrocarbon degradation. This is probably due to the mechanisms of interaction with toxic metals (e.g., biosorption and bioaccumulation) are faster than mechanisms involving the action of enzymes and biosurfactants for hydrocarbon remediation [75,103,129].

The measurement of the efficiency rate in mycoremediation is the main criteria to analyze the performance of bioinoculants, although this rate is influenced by several operational parameters; which are difficult to maintain under conditions appropriate to the bioinoculant during a real application [143,182]. In summary, high efficiency rates in mycoremediation are achieved through the use of bioinoculants under controlled conditions, mainly in liquid culture simulating remediation of aquatic environments. However, experiments in soil microcosms better mimics contaminated environmental conditions, since they simulate contaminant-fungus-soil interactions, which promotes results closer to real conditions [6,41]. Furthermore, technologies related to the use of bioinoculants in bioreactors to carry out mycoremediation or to produce metabolites for this purpose are constantly developing [32,144]. The next sections discuss in detail this information that concerns the bioprocessing and formulation of bioinoculants and strategies to improve their use in mycoremediation of hydrocarbons or toxic metals.

7.2 Bioreactors: controlled system configurations

Bioreactors are engineered equipment projected with control systems to ensure the maintenance of appropriate operating conditions for the process. Engineering principles such as mass transfer, mixing, aeration, etc., promote the versatility of different bioreactor configurations that can provide specific conditions related to the type of process in order to promote greater efficiency [1,196]. Fungal bioprocesses related to hydrocarbon and toxic metal remediation, when conducted in bioreactors, are to produce metabolites or analyze influence and behavior of these metabolites under different physicochemical and/or biological conditions during remediation [16,32]. Thus, mycoremediation processes for hydrocarbons or toxic metals have been carried out in submerged (water), semi-solid (soil sludge) or solid (air biofilters) state bioreactors [183,196,197]. The use of submerged culture bioreactors allows scale-up and process control, as well as being manageable, predictable, and easier to handle in a confined environment than *in situ* or solid phase developed systems [1,95].

Stirred tank reactors (STR) have mechanical stirrers that promote high shear stresses, which can cause fragmentation of the mycelium and/or pellet formation [95]. Higher phenol degradation was achieved in STR via laccases and other phenol-oxidases produced by *P. sajor-caju* compared to the same enzyme when produced in shaking flasks [27]. Differentially, air lift reactors are pneumatically agitated by air injection that impose low shear stress, which maintains the integrity of dispersing hyphae and mycelia and increases the surface contact area, requiring less energy compared to STR [1,95]. Air lift reactors have been used to scale up bioemulsifier production from *A. brasiliensis* applied to PAHs remediation [32]. A maximum removal at 92% of phenol (4.0 $mmol \bullet L^{-1}$) in liquid medium was achieved from laccases excreted by *P. sajor-caju* in STR, while in air lift reactor the maximum removal was 82% [27]. Moreover, STR and air lift were used for the development of a *Trichoderma viride* bioinoculant involved in Cr^{+6} biotransformation. A higher biotransformation capacity was achieved in air-lift reactor, since mechanical agitation in STR caused negative effects on the mycelium [196].

Slurry phase stirred tank reactor represent a highly engineered treatment system for soil remediation, since this system allows increased surface phenomena such as gas/liquid and solid/liquid mass transfer due to agitation [49,118]. Biotreatment in sludge-phase reactor can occur with the input of contaminated soil, water, air, fungal bioinoculant, nutrients and biosurfactants, while the output streams will be decontaminated soil, sewage, biomass, CO_2 and by-products [118]. The use of the bioinoculant *B. adusta* in sludge phase reactor promoted the degradation of several PAHs, probably due to the higher availability of oxygen and nutrients. This technology also maintains sludge homogeneity, which avoids solids separation during mycoremediation [183].

Bioreactor configurations such as packed bed, trickle bed and bioscrubbers are the main forms used as fungal biofilters for remediation of air containing volatile hydrocarbons [198,199]. Packed bed bioreactors have an absorption column configuration and can be multiphase; they also have inlet for contaminated air stream in upward or downward flow, which permeates the pores of the packing material where the fungus is immobilized, promoting the exit of a decontaminated air stream due to microbial degradation [4,200]. Trickle bed bioreactors have a configuration almost like the packed bed type; however, they are smaller and have spray nozzles on their top for intermittent addition and recirculation of nutrient solution and water. Furthermore, these reactors operate in countercurrent flow and can generate liquid waste [197,198]. The bioscrubber-type differ from the other biofilters previously mentioned only because their operation depends on a prior mass transfer step from the contaminant from the gas phase to a liquid phase that must be subsequently decontaminated [199]. In general, the efficiency of a biofilter-type bioreactor depends on the air inlet flow rate, nutrient starvation state and physicochemical properties of the packing material [156,200].

7.3 Nutritional Parameters

Fungal metabolism have its metabolic pathways altered according to the availability (presence or limitation) of substrates, so nutritional parameters such as carbon sources, nitrogen, trace elements, etc. must be supplied appropriately to ensure bioinoculant growth, which in turn influences the rate of hydrocarbon degradation [31,62,65]. For instance, supplementation with carbon sources is required during degradation of alicyclics by fungal bioinoculants, as these hydrocarbons do not support microbial growth due to their high recalcitrance [62]. The degradation of benzo[a]pyrene by the bioinoculant *Fusarium solani* increased approximately 9-fold when the carbohydrate carbon source (glucose) was replaced by a lipid substrate (olive oil) in liquid medium [65]. In addition, supplementation with nitrogen sources such as urea and ammonium dihydrogen phosphate increased engine oil degradation by 72% and 54% by the bioinoculant *A. sydowii*, respectively [31]. However, high concentrations of NPK nutrients can reduce microbial hydrocarbon degradation activities [1,31]. Supplementation of fungal bioinoculants with substrates such as citric acid, ferulic acid, glycerol, veratrole alcohol and copper sulphate can induce increased production of peroxidases and oxidoreductases in liquid cultures or soil microcosms [8]. Additionally, the bioinoculant *P. chrysosporium* modified its enzymatic mechanism involved in hydrocarbon degradation according to nutrient availability: the strain preferentially expressed peroxidases in nutrient-limited media and CYPs in nutrient-sufficient media [201].

The addition of lignocellulosic biomass to contaminated soil can enhance the performance of fungal bioinoculants as the biomass supports fungal growth, induces the excretion of oxidative enzymes and acts as a texturizing agent to enhance porosity and oxygen transfer [158,160,180]. When wood chips were added to the treatment of water contaminated with PAHs and phenols, laccases produced by *P. dryinus* showed a higher catalytic activity compared to those produced with glucose. However, the phenol concentrations were increased due to the depolymerization of lignin [179]. Furthermore, cometabolic substrates such as lignin and vanilla, when added to benzo[a]anthracene contaminated soils, promoted the stimulation of autochthonous fungi, which may cause hydrocarbon degradation [173,202]. Additionally, fungal bioinoculants that produce biosurfactants can have their productivity increased when there is a supplementation with a carbohydrate source, because the combination with the available hydrocarbon induces the excretion of biosurfactants [45].

7.4 Operational conditions and abiotic parameters

In general, abiotic parameters such as temperature, pressure, Eh, pH, moisture, salinity, aeration and mixing directly influence the performance of fungal bioinoculants, as these parameters affect microbial physiology regarding enzymatic pathways, balance of catalytic reactions, contaminant transport (desorption, diffusion) and excretion of metabolites [11,203]. Furthermore, these parameters can also affect biogeochemical processes; as well as influence the chemical structure, solubility, bioavailability and mobility of hydrocarbons, toxic metals and/or organic matter, during mycoremediation [57,108].

When the bioinoculant *A. sydowii* was subjected to high pressure reactors (10MPa) to check its ability to degrade spent motor oil under such conditions, this resulted in an increase of 11.3% compared to the degradation under mild pressure conditions (0.1MPa) [31]. This was explained by the higher aggregation and reduction in the size of mean diameter of hyphal filament under high pressure condition, which may have increased the surface area of active sites for hydrocarbon assimilation and degradation [31]. Moreover, high saline concentrations can negatively affect mycoremediation, as this condition tends to decrease solubility of enzymes and oxygen in aqueous solution, as well as decrease bioavailability of hydrocarbons [53]. Bioinoculants composed of halotolerants such as *L. tetrasporus* and *P. variotii* would be suitable for use in saline soils as they showed 38% and 31% efficiency for petroleum hydrocarbon degradation under high salinity (45 $g \bullet L^{-1}$) conditions, respectively [141].

The pH of soils contaminated with PAHs ranges from pH 3 to 9 [41,204]. Higher efficiencies of PAH degradation were reported at neutral pH, although generally fungal bioinoculants and enzymes can interact with PAHs under slightly acidic conditions (Table 1 and 2) [80,184]. However, *Talaromyces* sp. isolated from contaminated soils showed good performance in the degradation of aliphatic and aromatic hydrocarbons under alkaline conditions (pH=9), which are extreme conditions for other microorganisms [204]. Additionally, alkaline pH induce the formation of negatively charged groups in the fungal biomass, which favors the biosorption of cationic metals [99].

The Eh measures the oxidizing (Eh positive) or reducing (Eh negative) capacity of the microenvironment in which the biotransformation of contaminants occurs, and influences biochemical mechanisms involved in the action of bioinoculants and/or enzymes applied to mycoremediation [137]. In addition, the moisture/water activity of the environment influences fungal growth, excretion of metabolites and biochemical reactions involved in mycoremediation, as these depend on a specific water level [59,129]. Addittionally, low temperatures can increase the viscosity of hydrocarbons and inhibit fungal enzymes activities involved in their degradation, although oxygen solubility is favored at low temperatures for aerobic degradation [57,178]. Higher temperatures increase the bioavailability and solubility of

hydrocarbons [197], and increase the capacity of fungal bioinoculants for biosorption due to increased activation of adsorption surfaces and diffusivity of toxic metals [99]. Trials on mycoremediation of hydrocarbons and toxic metals are commonly conducted at temperatures between 20 and 32 °C (Table 1 and 2).

The agitation is a parameter that considerably influences the production/application of fungal bioinoculant and metabolites for environmental remediation [67,205]. Bioinoculants composed of filamentous fungi generally require high oxygen demand and the minimization of mechanical disturbance for real application in soil remediation [101,150]. Under static conditions, air biofilters inoculated with filamentous fungi may exhibit hydraulic/gas retention or loss in interstitial fluid volume due to overgrowth of hyphae, which also promotes higher pressure drop [199]. Regarding to enzymatic mycoremediation, *Polyporus* sp. produced ligninolytic enzymes and degraded chrysene about 2 times more efficiently when orbitally shaken than in a stationary culture [67]. This was probably due to fungus morphology that in the form of pellets increases the mass transfer between cells and medium, a condition not met in the stationary culture characterized by the formation of a mycelial layer on the substrate surface [67]. Nevertheless, in relation to biosurfactant application, those produced by *Y. lipolytica* removed 98% of engine oil when applied under agitation, as opposed to 30% under static conditions [206]. Likewise, biosurfactants from *C. tropicalis* were able to remove Zn (80%), Cu (70%) and Pb (15%) when applied under orbital mixing conditions and at double CMC. However, under static and at normal CMC, its capacity was reduced considerably: Zn (32%), Cu (20%), and Pb (0.5%) [205].

7.5 Downstream processing: efficiency, ecotoxicological safety, recovery and formulation

The development of fungal bioprocesses in environmental remediation involving the production or application of bioinoculants may include downstream processing to analyze their efficiency and ecotoxicological safety, as well as to propose recovery and formulation of bioinoculants on a market scale.

Under laboratory conditions, the efficiency of bioinoculants in remediation of hydrocarbons and toxic metals are measured using high resolution chemical techniques such as GC-MS and AAS, respectively [34,41]. Lower resolution techniques such as gravimetry and respirometry also present less accurate quantitative data on hydrocarbon remediation efficiency [128,153]. However, the efficiency of bioinoculants is certainly impossible to measure in a real application due to the complexity and dynamics of soil-fungus-contaminant interactions along small interfaces of the contaminated environment, as well as different distributions of contaminant concentration gradients may imply inconsistent results [108].

Exotic fungal bioinoculants may pose risks, such as decreasing microbial and plant biodiversity in a natural habitat, due to their behavior as an invasive species [207]. Fungal bioproducts must not promote an additional ecotoxicological risk for contaminated environments after their application, i.e., they must not affect the metabolism of terrestrial or aquatic plants and animals [71,116]. Some fungi can excrete intermediate metabolites such as oxygenated, alkylated and nitro-PAHs that are formed during enzymatic degradation of PAHs and are more toxic than their original counterpart [4,76]. Thus, technologies to verify the ecotoxicological safety via mutagenicity, phytotoxicity and genotoxicity tests on the use of bioinoculants or biosurfactants in mycoremediation should be based on fast growing organisms that are very sensitive to toxic chemicals [35,41,71,116,205]. For example, vegetables like watercress (*Lepidium sativum* L.), cabbage (*Brassica oleracea*), beans (*Vigna radiata*); animals like fish (*Poecilia vivipara*) and shrimp (*Artemia salina*); and bacteria like *Vibrio fischeri* (bioluminescence test) and *Salmonella typhimurium* (AMES test) are organisms that allow an easy response in environmental toxicology assays [35,41,71,116,205].

Downstream processing technologies such as recovery, purification, and characterization in developing bioinoculants do not require high resolution techniques presumably due to their environmental purpose, thus purification steps are not necessarily required. Thus, filamentous and yeast bioinoculants can be adequately recovered by unit operations involving solid/liquid separation such as filtration and centrifugation [41,185]. However, the application of drying techniques can increase the storage feasibility and shelf life of metabolically inactive bioinoculants compared to those with active metabolism.

Although the formulation and use of fungal bioinoculants in environmental remediation is not yet consolidated for a practical application, the technologies related to this formulation are predictably similar to those required for formulation of bioinoculants used in agriculture, preferably with minimal unit operations of preparation [208]. Besides containing nutrients (C, N, P and K), the formulation of fungal bioinoculants may require the addition of preservatives (e.g., potassium sorbate), stabilizers (e.g., hydroxyethyl cellulose) and chelating agents (e.g., ethylenediaminetetraacetic acid, except metal ions) similarly to the formulation of bioproducts containing fungal biosurfactants [23,116]. Bioinoculants can be formulated in liquid state, which promotes greater ease for application, as well as in solid state through the use of carriers (e.g., polymers) to provide stabilization and protection of the strain during transport, storage and application [208]. The solid carriers involved in formulation of fungal bioproducts are related to immobilization technology, which is discussed below.

7.6 Immobilization: cells, metabolites and nanotechnology

Immobilization systems for fungal bioinoculants and/or enzymes increase the mycoremediation performance in comparison to free cells as immobilized cells have their cell density, mechanical strength and structural rigidity increased [6,84,176]. In other words, the immobilization process alters mass transfer between contaminant-bioproduct, which can generate a microenvironment possibly more favorable for remediation [20,73]. Furthermore, immobilization supports can also positively favor other or sequential mycoremediation steps such as solid-liquid separation and/or support regeneration for reuse [60,176].

Organic supports composed of fungal polysaccharides and pigments present potential to immobilize bioinoculants or hydrocarbons/metals [30,209]. For example, the immobilization of the biosurfactant-producing bioinoculant *C. tropicalis* in chitosan and its adsorption on a biofilm formed on gravel increased the degradation of diesel oil by 22% since the polymer favored the fungal metabolism [209]. Similarly, the polymer (1→3)-α-d-glucans extracted from *Lentinus edodes* showed sorption capacity for Ni^{2+} (11%), Cd^{2+} (24%), Zn^{2+} (4%) and Pb^{2+} (72%) in aqueous solution due to the interaction of these metals with oxygen-containing functional groups of the biopolymer [210]. The use of semipermeable cellulose membrane to immobilize *A. nidulans* by encapsulation also increased approximately 3.6-fold the capacity for biosorption/desorption of copper in aqueous solution by multiple adsorption-desorption cycles compared to free cells of this strain [176].

Porous inorganic supports contribute to a better physical retention of bioinoculants and/or metabolites in their structures, which allows better contact between contaminant and immobilized cell without changing the physicochemical and thermodynamic properties of the process [6,211]. Several packing materials such as ceramic pellets [197], vermiculite [16], perlite [200] and polyueratan [156] have been used to immobilize fungal cells for aromatic and/or aliphatic hydrocarbon degradation. *Fusarium solani* reduced by 18% the total PAH in soil microcosms when immobilized on expanded clay particles [6]. *Trichoderma longibrachiatum* promoted a microbial biofilm formation that removed about 70% of phenanthrene in soils when immobilized on nylon sponge [211]. Immobilization of *S. cerevisiae* on cross-linked gel in boric acid-saturated calcium alginate also allowed maximum biosorption of radioactive uranium (113.4 $\mu mol \bullet g^{-1}$), besides intensifying the adsorption on both the surface and internal parts of the yeast [20]. Additionally, mineral particles as support for intracellular immobilization of metal ions in *S. cerevisiae* [98] and *P. chrysosporium* [97] enhanced the removal of toxic metals in aqueous solution, since these strains, when functionalized with calcium carbonate ($CaCO_3$) in their internal structure, achieved higher rates (3 to 4x) for immobilization of Pb^{2+} and Cd^{2+} compared to untreated strains [97,98].

Enzyme immobilization can enhance the biodegradation of hydrocarbons because it promotes for greater enzyme stability, resistance to proteolysis, increased catalytic activities and enzyme recyclability and shelf life [1,73]. Laccases from *T. versicolor* immobilized on Fe and Al soil minerals showed increased catalytic activities compared to free laccase at low pH, which favors their application for soil remediation [84]. Covalent immobilization of laccase produced by *Myceliophthora thermophila* on support (silica) functionalized with epoxy promoted degradation of phenolic compounds derived from oxidation of PAHs, such as catechol (95% in 2h) and phenol (13% in 24h), as well as improved enzyme stability at different temperature profiles and organic solvents in comparison to free enzymes [73]. Membranes fabricated by emulsion electrospinning, composed of structural nanofibers in the shell (for contaminant adsorption) and with immobilized laccase in its core (for contaminant degradation) and pores in its shell (for mass transfer), promoted enhanced adsorption and removal of hydrocarbons in soils [212].

Nanotechnology has allowed achieving higher performance in metal immobilization using nanoparticles synthesized from fungal cells and/or fungal metabolites as reducing agents for biogenic synthesis of such miconanoparticles [96,213]. Nanoparticles as metal immobilization support have high surface/volume ratio, which confers a higher expected number of ligands for metal sorption [96,213]. Nanoparticles synthesized from chitosan extracted from *C. elegans* and sodium tripolyphosphate ($Na_5P_3O_{10}$) showed better potential to immobilize Pb^{2+} than Cu^{2+} in contaminated soil or water [96]. Silver nanoparticles biofabricated/adsorbed on the carbonized mesoporous surface of *Aspergillus foetidus* increased approximately 3-fold the arsenic biosorption compared to the same fungal biosorbent without the nanoparticles [213].

8 EXPERIMENTAL PROCESS DESIGN: A PROPOSAL FOR INTEGRATED TECHNOLOGIES

Although integrated technologies are even more challenging in a practical use, they are under constant development to overcome technological limitations and increase the efficiency of mycoremediation [52,214]. A combination of technologies involving production and application of bioinoculants or metabolites can extend the allocation of resources appropriately to fill small gaps that may still limit remediation, as well as increase the amount of resources available to better carry out the process [106,215].

8.1 Physicochemical-biological systems

Bioinoculants or metabolites when integrated into physicochemical technologies have promoted superior results compared to technology without such fungal bioproducts [52,214]. The electrokinetic remediation is a technology indicated for soils with low hydraulic permeability, in which it would be unfeasible to use fungal bioinoculants due to restrictions related to low

oxygen availability and permeation of its hyphae [146,214]. However, the combination of SL with electrokinetic remediation enhanced the removal of Cu (53.2%), Zn (62.0%), Cr (53.0%), Pb (52.4%), Ni (56.2%) and Fe (37.0%) due to the chelating agent action of the biosurfactant in acidic conditions, which allowed greater mobility of toxic metals through electro-migration and electro-osmosis during electrokinetic remediation [214]. Similarly, the use of SL (20 $g \bullet L^{-1}$) increased Cd removal by 71.2% when combined simultaneously with an ultrasound technique (35 kHz) and temperature control (50°C) during soil washing, as cavitation and high temperature promotes greater dispersion and molecular movement of the metal, respectively [216].

The oxidative process of ozonization promotes degradation of hydrocarbons by addition of ozone. The combination of this technology with the use of fungal bioinoculants can increase efficiency for the degradation of these contaminants [52]. After the mycoremediation of soils contaminated with diesel by *T. harzianum*, the application of ozone in these soils increased diesel degradation by two-fold compared to the only ozonation process. It is important however to mention that lower degradation rates were achieved after ozone application due to the antimicrobial activity of ozone [52].

8.2 Multiple biosystems

We define multiple biosystem applied to mycoremediation of hydrocarbons and toxic metals as any bioprocess involving the (1) combination of metabolite and bioinoculant, (2) a system composed of a single fungus producing multiple metabolites, the (3) combination of different metabolites and the (4) combination of bioinoculants or metabolites with other biological systems, such as plants, animals or other microorganisms.

The sequential combination of bioleaching (by organic acids) and biosorption can be an alternative to enhance the remediation of toxic metals since the bioinoculant *A. niger* promoted the solubilization of Pb and Zn during its cultivation in liquid medium (bioleaching) and its biomass was considered a good biosorbent for such metals when applied later [106].

The production of multiple metabolites such as enzymes and biosurfactants by a single fungus can occur because the substrate can be directed to different metabolic pathways of the strain that are activated in its presence. This can favor the economy of production process and efficiency of the process for simultaneous application of these two metabolites [129,186,189]. *Pleurotus florida* degraded crude oil (55%) in liquid medium by simultaneous production of biosurfactants and intracellular and extracellular enzymes such as tyrosinase and laccases, respectively [71]. It

is speculated that positive enzyme-biosurfactant synergy in hydrocarbon degradation relates to increased enzyme activity due to the higher bioavailability of the contaminant promoted by biosurfactants [71]. However, high concentrations of surfactants can also reduce the enzymatic activity [50]. Additionally, due to nutritional and environmental parameters that can divert and/or induce towards other metabolic pathways, the production of multiple metabolites for mycoremediation is still underdeveloped [129].

The combination of different classes of biosurfactants can increase washing efficiency of petroleum sludge, as binary system composed of non-ionic SL and anionic (bacterial) lipopeptide promoted the formation of mixed micelles with superior heterogeneous and structural properties. These include lower CMC and better efficiency in reducing surface and interfacial tension compared to single surfactant system [54].

Commercial SL, when combined with phytoremediation processes (remediation by plants) of Cd by pot-grown *Bidens pilosa*, promoted higher bioaccumulation of this metal in roots (5.36 $\mu g \bullet pot^{-1}$) and shoots (15.34 $\mu g \bullet pot^{-1}$) of the plant, as their addition promoted higher metal availability [48]. Commercial SL, can also when applied to bacterial remediation, increased the degradation of petroleum hydrocarbons in soil microcosms by 13% compared to bacterial remediation alone. This maybe the results of the fungal surfactants that may have biostimulated the bacteria activities [47]. The combination of fungal bioinoculants with phytoremediation and/or vermiremediation (use of earthworms) are promising technologies for hydrocarbon/or toxic metal remediation [48,190,217].

Synergistic plant-fungus interaction can promote microbial growth due to secretion of root exudates (phenolic compounds, organic acid), while plants can obtain mineral nutrients and increase their defense potential due to fungal action [42,190,218]. Bioinoculants composed of the AMF *G. versiforme* increased Cd bioaccumulation in shoots and roots of *Solanum nigrum*, as well as increased plant growth and resistance to metal toxicity [36]. Another good example is the bioinoculant *P. chrysosporium*, which increased by almost two-fold the phytoremediation efficiency by *Amaranthus hypochondriacus* in soils co-contaminated with hydrocarbons and toxic metals, an even further two-fold greater efficiency was achieved when biosurfactants were added to this process [218]. The interaction between *S. nigrum* and *M. circinelloides* reduced oxidative toxicity of Pb, Cd and As and allowed to alter the mobility of these metals through biosorption and bioaccumulation mechanisms, besides increasing plant growth [42]. In addition, microbial-plant remediation carried out by the bioinoculant *C. laeve* and *Salix viminalis* increased pyrene degradation compared to the independent processes of mycoremediation (14%) [190].

The bioavailability of organic contaminants and toxic metals to fungal strains and plant roots can be increased through the use of earthworms in soils. These animals increase porosity and modify soil physicochemical parameters, which in turn favors the remediation of the soil due to the greater contact surface reached [37,219]. Although not necessarily reported for petroleum hydrocarbons and toxic metals, mechanisms for remediation of organic contaminants by vermiremediation include vermiacumulation, vermiextraction, vermitransformation and drilodegradation [219]. Multiple biosystems consisting only of the combination of vermiremediation and mycoremediation are still unexplored, so are those combined with other technologies such as phytoremediation [37,217].

The combination of vermirremediation using *Eisenia fetida* with a multiple biosystem composed of *P. chrysosporium* and *Medicago sativa* degraded higher levels of phenanthrene (11%) in soils compared to the multiple biosystem only [217]. Likewise, although the individual potential of each remediation system was not evaluated, the use of the AMF *Rhizophagus intraradices* with *S. nigrum* with *E. fetida* reduced the initial Cd concentration in soils by 8% compared to the untreated soils (120 $mg \bullet kg^{-1}$ of Cd) [37]. The application of microbial remediation technologies combined with vermiremediation is still limited to low concentrations of hydrocarbons due to toxicity issues [219,220]. Baykal-EM© is a mixed bioinoculant composed of fungi (*Saccharomyces*, *Aspergillus* and *Penicillium*), photosynthetic bacteria (Thiorhodaceae, Athiorhodaceae and Chlorobacteriaceae), nitrogen-fixing bacteria (*Azotobacter* and *Clostridium*) and Actinomycetales [220]. This biotechnological product Baykal-EM©, when combined with annelids *E. fetida*, *Eisenia andrei* or *Dendrobena veneta*, achieved superior results for hydrocarbon degradation than when without the animals [220]. However, the biotic potential of all these annelids at different hydrocarbon concentrations was decreased, which presumes that the microbial consortia may facilitate oil uptake in the digestive pathway of the earthworm, which in turn increases the toxic effects of the contaminants [220]. Furthermore, ethical aspects and both environmental and ecological safety related to the use of animals (even invertebrates) discourage real application of the combination of myco- and vermiremediation.

8.3 Mixed fungal bioinoculants: consortium with other fungi, bacteria or microalgae

Although axenic fungal bioinoculants have demonstrated high efficiency in remediating hydrocarbon and toxic metals, they may have their enzymatic mechanisms limited to the degradation of specific/different fractions of petroleum hydrocarbons [62,181,221]. Furthermore, it is very likely that in a practical application their use will predictably not be under axenic conditions.

Alternatively, mixed cultures of fungi and/or bacteria can enhance efficiency/productivity and reduce the period required to achieve maximum hydrocarbon and toxic metal removal compared to axenic cultures [150,161], particularly due to the combination of different genotypes and diversity of metabolic processes of the strains [152,153]. For example, co-culture of *Acinetobacter* sp. and *Scedosporium* sp. increased the abundance of genes involved in aliphatic degradation such as alkane monooxygenase (*alkB*) and cytochrome P450 alkane hydroxylase (*CYP52*) by 43% and 65% compared to axenic cultures, respectively. This fact was attributed to biosurfactant excretion by *Acinetobacter* sp., which increases hydrocarbon bioavailability [153]. Furthermore, the use of co-cultures of biosurfactant-producing yeasts such as *Sarocladium* sp. and *Cryptococcus* sp. Promoted greater degradation of pyrene in a shorter time than with individual strains [111].

The symbiotic and co-metabolism relationships between fungi and bacteria that compose mixed bioinoculants can enhance hydrocarbon mycoremediation [19,204]. Mixed bioinoculant composed of *Talaromyces* sp. And *Acinetobacter baumannii* degraded different fractions of petroleum hydrocarbons, as mostly n-alkanes were degraded by the fungus, while aromatics and branched alkanes were better degraded by the bacteria [204]. In addition, some bacterial bioinoculants produce exopolysaccharides as a secondary carbon source, which supports the growth of fungal strains [222]. Mycelia can also mobilize contaminants through cytoplasmic transport linked to 'hyphae pipelines' vesicles, acting as vectors for dispersal and transport of contaminant-degrading bacteria [101,153].

Syntrophic action between different microbial strains complements metabolic reactions involved in hydrocarbon degradation when a single strain is unable to perform degradation without the cooperation of others, as the previously formed metabolite is a substrate for next catalysis [62,181]. For example, alicyclics are only degraded by mixed bioinoculants, since the enzyme cascade involved in this degradation is almost never found in the same strain [5,62]. The microbial consortia composed of eight filamentous fungi, three yeasts and four bacteria, when inoculated sequentially (bacteria added after one week), promoted greater degradation of crude oil [19], pyrene and tetracosane [154] compared to consortia composed separately of only fungal or bacterial strains, as well as when compared to the concomitant inoculation of all microorganisms at the start of the process. Thus, enzymes produced by fungal bioinoculants produce more soluble and less toxic intermediate metabolites from hydrocarbon degradation, which in turn can be further degraded by bacterial bioinoculants [19,154].

Overall, microbial consortia of fungi and/or bacteria can enhance: enzyme and biosurfactant production [152,204], bioaugmentation processes [41], air biofiltration [16] and metal mobility [55,221] during mycoremediation. Fungal consortia consisting of *Alternaria alternata*,

Aspergillus terreus, *C. sphaerospermum*, *Eupenicillium hirayamae* and *P. variotii* degraded diesel oil and increased enzymatic activity of laccase, manganese peroxidase, lignin peroxidase and catalase compared to using individual strains alone [152]. Bioaugmentation with autochthonous fungi belonging to the genus *Penicillium*, *Ulocladium*, *Aspergillus* and *Fusarium* increased removal of petroleum hydrocarbons by 65% compared to biostimulation in soil microscosms [41]. Additionally, the mixed bioinoculant composed of *F. solani* and *Rhodococcus erythropolis* removed approximately 60% of toluene and benzo[a]pyrene in an upflow air biofilters [16]. *Fusarium solani* predominantly colonized the first stage of the biofilter and *R. erythropoli* preferentially colonized the second and third stages, probably due to the feeding with mineral medium that occurred through the top of the reactor. This demonstrated that these different strains grew differently in the presence of high flow of contaminated air according to nutrient availability and carried out toluene and benzo[a]pyrene removal [16].

Regarding to toxic metals, fungal consortia composed of *Aspergillus niveus*, *A. flavus* and *A. niger* increased (on average) biosorption efficiency of Zn (20%), Pb (10%), Cd (24%) and Ni (16%) in aqueous solution compared to individual strains [144]. The fungal consortia of ascomycetes (*Aspergillus*, *Fusarium*, *Penicillium*, *Purpureocillium*, etc.) and basidiomycetes (*Phanerochaete*, *Polyporales*, *Perenniporia*, etc.) also enhanced bioaccumulation of Ni and Pb in soil microcosm since ascomycetes are known to have a potential as biosorbents, while basidiomycetes are known for the production of oxidative enzymes [221]. Moreover, mixed bioinoculant composed by the biosurfactant-producing yeast *Meyerozyma guilliermondii* and organic acid-producing bacteria *Acidithiobacillus* promoted the solubilization of Zn (76.5%), Ni (59.8%), Cu (22.0%), Cd (9.8%), Cr (99. 8%) and Pb (7.1%) in sewage sludge, since this microbial consortium promoted better bioleaching due to excretion of organic acids and biosurfactants that alter metal mobility [215].

The formulation of a mixed bioinoculant for hydrocarbon and toxic metal remediation requires that there is no interspecies inhibition for the survival of each strain, as well as that there is temporal stability of the microbiota, which can be achieved through efficient substrate availability and transport [153,160]. In this context, the use of *C. laeve* bioinoculant in PAH-contaminated soil increased the relative abundance of bacteria (*Rhizobium* and *Bacillus*) and fungi (*Hypocreales*, *Mortierellales*, *Mucorales*, and *Pezizales*) that may be involved in the degradation of this contaminant. However, this bioinoculant also antagonized other putative PAH-degrading bacteria [190]. Furthermore, although *T. longibrachiatum* is a promising bioinoculant for the remediation of phenanthrene-contaminated soil, bacteria native to this soil, such as *Sphingomonas*, *Sphingobacterium*, *Acidovorax*, *Massilia*, *Flavobacterium*, *Cupriavidus* and *Aeromicrobium*, enhanced the degradation of this contaminant. Prior sterilization of the

contaminated soil decreased degradation by 82% [40]. Hence, interactions between bioinoculant strains [190], nutrient availability [153] and the native microbial communities [40] influenced the efficiency of hydrocarbon degradation via mixed bioinoculants with respect to enhancing or retarding strain development.

Although bacterial-microalgae consortia have shown promising results for the remediation of petroleum hydrocarbons [223]; the application of fungal-microalgae consortia to degrade such hydrocarbons is little explored compared to the use of fungi to enhance bioflocculation processes for microalgae harvesting [224,225]. Regarding hydrocarbon degradation, the consortium between the microalgae *Desmodesmus* sp. and the fungus *Rhizopus* sp. showed a superior removal of phenol (25 mg•L^{-1}) in aqueous solutions of 25% and 29% compared to axenic cultures of these microorganisms, respectively [225]. Applied to the remediation of toxic metals, the microbial consortia of the microalgae *Chlorella vulgaris* and *A. niger* had a similar result to the use of only this fungus for the removal of Cd in aqueous solutions [29]. However, the aqueous solutions from this consortium had its pH decreased during metal removal due to the excretion of organic acids by the fungus, promoting metal bioleaching [29]. Possible synergistic relationships between fungi and microalgae during hydrocarbon remediation may include the additional supply of organic carbon source (exopolymers) and oxygen produced by microalgae for fungal metabolism [223]. In return, microalgae assimilate inorganic carbon as CO_2 released by the fungal aerobic respiration, or organic carbon produced from the degradation of complex substrates via fungal enzymes [223]. Furthermore, the adherence and interaction between the cell surfaces of microalgae and fungi can promote a favorable microenvironment for hydrocarbon and toxic metal remediation, as there is an increase in the contact surface area, which also increases hydrocarbon uptake and metal sorption [223].

9 FINAL CONSIDERATIONS

Fungi are potential prototypes to be used in the biotechnological and environmental development of remediation processes for hydrocarbons and toxic metals. This is due to the metabolism, physiology and morphology of several fungi that make them able to interact with contaminants and/or to produce metabolites possibly scalable to an industrial production focused on the commercialization of fungal bioproducts for environmental remediation. Microbial enzymes (added chemical surfactants, nutrients) (http://www.osei.us/) and fungal biosurfactants (https://www.allied-c-s.co.jp/acs-sophor) are already produced and marketed on a large scale (≈ 1000L) for environmental remediation. However, technological issues regarding to the direct application, storage and shelf life of these products require further research to enable the use of these technologies, mainly related to enzymatic mycoremediation. Fungal

biosurfactants show promising results for washing contaminated soils even when they are used directly from the fermented liquid culture, without any purification step. Furthermore, although the potential of various fungal bioinoculants in the remediation of hydrocarbons and toxic metals is widely demonstrated under controlled conditions, their real application in contaminated environments remains, analogously to sporulated fungi, awaiting appropriate conditions for development, i.e., technical and economic feasibility for proper consolidation.

Among the fungal metabolites studied by the scientific community with focus on environmental remediation, enzymes and biosurfactants have received more attention for hydrocarbon remediation, while other metabolites such as pigments, organic acids, polymers or bioinoculant itself (due to cell wall composition) for toxic metals remediation. It is assumed that bioprocesses involving bioprospecting of fungal isolates as possible bioinoculants and/or producers of enzyme or biosurfactants for environmental remediation can take years to obtain an effective and accurate answer about the potential of the isolates; since several isolates must undergo a combination of screening techniques and be molecularly characterized to ensure biosafety for handling in bioprocesses. In this regard, enzymes have received a little more attention than biosurfactants in studies on bioprospecting and integration of molecular biology techniques and bioprocess engineering for mycoremediation of hydrocarbons and toxic metals.

Nutritional and operational parameters, when adequately supplied, can minimize negative effects on fungal metabolism to achieve maximum performance through the full utilization of available resources provided by the natural archetype of fungal bioinoculants for environmental remediation. However, only from bioengineering of fungal strains is it possible to extrapolate the maximum performance achieved via maintenance of process parameters. Thus, genetic engineering has allowed the heterologous production of metabolites with higher yield or superior physicochemical-biological properties compared to non-engineered fungi. Moreover, -omics technologies and bioinformatics tools have enabled the scientific elucidation of gaps to promote more efficient mycoremediation processes, mainly on: a) regulatory mechanisms (induction/repression) involved in enzyme and biosurfactant production; b) contaminant degradation pathways; and c) metabolic responses of fungal strains under multiple pollutant conditions.

Other emerging technologies to increase performance in mycoremediation are related to immobilization techniques and biogenic synthesis of nanoparticles that promote enhancements in structural and mechanical characteristics in bioinoculants and/or metabolites, which in turn favor their interaction with contaminants and mass transfer during remediation. Integrated technologies based on the combination of bioinoculants and/or fungal metabolites with physico-chemical remediation, such as ozonation or electrokinetic techniques, and the combination with

other bioremediation methods such as phytoremediation, vermiremediation or mixed microbial bioinoculants have achieved superior results compared to the use of bioinoculants or metabolites alone in most cases. Furthermore, the simultaneous use of multiple metabolites such as enzymes and biosurfactants, or the combination of different classes of biosurfactants, can promote synergistic actions during remediation to achieve better performance since biosurfactants increase the bioavailability of contaminants for oxidative enzymatic action, as well as micelles with superior properties can be formed by mixed biosurfactants.

This review has not been restricted to remediation of specific types of hydrocarbons and toxic metals and/or potential fungal bioproducts, which has broadened the coverage of different technologies to be applied in general. Although such technologies related to bioprocess and biomolecular engineering techniques may be trivial to any type of bioinoculant, metabolite or contaminant within its class, some of these technologies/methodologies discussed herein may not be applied to other bioprocesses due to their particularities, as well as divergence in operational parameters may decrease the coverage range or influence the response on the use of such technology. However, the high number of articles screened from the last twenty years of our survey brings robustness to the data discussed here, as well as relevance to the promotion of fungal bioprocesses for remediation of different hydrocarbons, toxic metals and/or possibly other emerging contaminants.

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Compliance with ethical standards

Conflict of interest

The authors declare no financial or commercial conflict of interest.

References

1. Pandey KA, Gaur VK, Udayan A. *et al.* (2021) Biocatalytic remediation of industrial pollutants for environmental sustainability : Research needs and opportunities. Chemosphere 272:129936. https://doi.org/10.1016/j.chemosphere.2021.129936

2. Magalhães KM, Carreira RS, Rosa Filho JS. *et al*. (2022) Polycyclic aromatic hydrocarbons (PAHs) in fishery resources affected by the 2019 oil spill in Brazil: Short-term environmental health and seafood safety. Mar Pollut Bull 175:113334. https://doi.org/10.1016/j.marpolbul.2022.113334

3. Bevitório LZ, da Silva NG, Pirovani JCM. *et al*. (2022) Impacts of tailings of Fundão dam (Brazil) rupture on marine fish: Metals bioaccumulation and physiological responses. Mar Pollut Bull.;177:113511.

https://doi.org/https://doi.org/10.1016/j.marpolbul.2022.113511

4. Prenafeta-Boldú FX, de Hoog GS, Summerbell RC (2019) Fungal Communities in Hydrocarbon Degradation. Cham: Springer International Publishing. https://doi.org/10.1007/978-3-319-60063-5_8-2

5. Varjani SJ (2017) Microbial degradation of petroleum hydrocarbons. Bioresour Technol 223:277–86. https://doi.org/https://doi.org/10.1016/j.biortech.2016.10.037

6. Fayeulle A, Veignie E, Schroll R. *et al.* (2019) PAH biodegradation by telluric saprotrophic fungi isolated from aged PAH-contaminated soils in mineral medium and historically contaminated soil microcosms. J Soils Sediments 3056–3067. https://doi.org/10.1007/s11368-019-02312-8

7. Singh PC, Srivastava S, Shukla D. *et al.* (2018) Mycoremediation Mechanisms for Heavy Metal Resistance/Tolerance in Plants. In: Prasad, R (eds) Mycoremediation and Environmental Sustainability. https://doi.org/10.1007/978-3-319-77386-5_14

8. Akhtar N, Mannan MA (2020) Mycoremediation: Expunging environmental pollutants. Biotechnol Reports 26:e00452. https://doi.org/10.1016/j.btre.2020.e00452

9. Chormare R, Anil M (2022) Environmental health and risk assessment metrics with special mention to biotransfer , bioaccumulation and biomagnification of environmental pollutants. Chemosphere 302:134836. https://doi.org/10.1016/j.chemosphere.2022.134836

10. Teke G, Hubai K, Diósi D. *et al*. (2020) Assessment of Foliar Uptake and Accumulation of Airborne Polyaromatic Hydrocarbons Under Laboratory Conditions. Bull Environ Contam Toxicol 104:444–8. https://doi.org/10.1007/s00128-020-02814-z

11. Daccò C, Girometta C, Asemoloye MD. *et al.* (2020) Key fungal degradation patterns, enzymes and their applications for the removal of aliphatic hydrocarbons in polluted soils: A review. Int Biodeterior Biodegradation 147:104866. https://doi.org/10.1016/j.ibiod.2019.104866

12. Quintella CM, Mata AMT, Lima LCP (2019) Overview of bioremediation with technology assessment and emphasis on fungal bioremediation of oil contaminated soils. J Environ Manage 241:156–66. https://doi.org/10.1016/j.jenvman.2019.04.019

13. Ross IS (1975) Some effects of heavy metals on fungal cells. Trans Br Mycol Soc 64:175–93. https://doi.org/10.1016/S0007-1536(75)80101-X

14. Davies JS, Westlake DWS (1979) Crude oil utilization by fungi. Can J Microbiol 25:146–56. https://doi.org/10.1139/m79-023

15. Leahy JG, Colwell RR (1990) Microbial degradation of hydrocarbons in the environment. Microbiol Rev 54:305–315 https://doi:10.1128/mr.54.3.305-315.1990.

16. Vergara-Fernández A, Yánez D, Morales P. *et al*. (2018) Biofiltration of benzo[A]pyrene, toluene and formaldehyde in air by a consortium of Rhodococcus erythropolis and Fusarium solani: Effect of inlet loads, gas flow and temperature. Chem Eng J 332:702–10. https://doi.org/10.1016/j.cej.2017.09.095

17. Benguenab A, Chibani A (2020) Biodegradation of petroleum hydrocarbons by filamentous fungi (Aspergillus ustus and Purpureocillium lilacinum) isolated from used engine oil contaminated soil. Acta Ecol Sin 416-23. https://doi.org/10.1016/j.chnaes.2020.10.008

18. Njoku KL, Akinyede OR, Obidi OF (2020) Microbial Remediation of Heavy Metals Contaminated Media by Bacillus megaterium and Rhizopus stolonifer. Sci African 10:e00545. https://doi.org/10.1016/j.sciaf.2020.e00545

19. Ghorbannezhad H, Moghimi H, Dastgheib SMM (2018) Evaluation of heavy petroleum degradation using bacterial-fungal mixed cultures. Ecotoxicol Environ Saf 164:434–9. https://doi.org/10.1016/j.ecoenv.2018.08.046

20. Chen C, Hu J, Wang J (2020) Biosorption of uranium by immobilized Saccharomyces cerevisiae. J Environ Radioact 213:106158. https://doi.org/10.1016/j.jenvrad.2020.106158

21. Damodaran D, Vidya Shetty K, Raj Mohan B (2013) Effect of chelaters on bioaccumulation of Cd (II), Cu (II), Cr (VI), Pb (II) and Zn (II) in Galerina vittiformis from soil. Int Biodeterior Biodegrad 85:182–8. https://doi.org/10.1016/j.ibiod.2013.05.031

22. Wollenberg A, Kretzschmar J, Drobot B. *et al*. (2021) Uranium(VI) bioassociation by different fungi – a comparative study into molecular processes. J Hazard Mater 411:125068. https://doi.org/10.1016/j.jhazmat.2021.125068

23. da Silva IGS, de Almeida FCG, Silva FCPR. *et al*. (2021) Application of Green Surfactants in the Remediation of Soils Contaminated by Hydrocarbons. Processes 9:1–21. https://doi.org/10.3390/pr9091666

24. Baranger C, Pezron I, Lins L. *et al*. (2021) A compartmentalized microsystem helps understanding the uptake of benzo [a] pyrene by fungi during soil bioremediation processes. Sci Total Environ 784:147151. https://doi.org/10.1016/j.scitotenv.2021.147151

25. Xiao X, Luo S, Zeng G. *et al* (2010) Biosorption of cadmium by endophytic fungus (EF) Microsphaeropsis sp. LSE10 isolated from cadmium hyperaccumulator Solanum nigrum L. Bioresour Technol 101:1668–74. https://doi.org/10.1016/j.biortech.2009.09.083

26. Saika A, Koike H, Yamamoto S. *et al*. (2017) Enhanced production of a diastereomer type of mannosylerythritol lipid-B by the basidiomycetous yeast Pseudozyma tsukubaensis expressing lipase genes from Pseudozyma antarctica. Appl Microbiol Biotechnol 101:8345–52. https://doi.org/10.1007/s00253-017-8589-6

27. Bettin F, Cousseau F, Martins K. *et al*. (2019) Phenol removal by laccases and other phenol oxidases of Pleurotus sajor-caju PS-2001 in submerged cultivations and aqueous mixtures. J Environ Manage 236:581–90. https://doi.org/10.1016/j.jenvman.2019.02.011

28. Ferreira INS, Rodríguez DM, Campos-Takaki GM. *et al*. (2020) Biosurfactant and bioemulsifier as promising molecules produced by Mucor hiemalis isolated from Caatinga soil. Electron J Biotechnol 47:51–8. https://doi.org/10.1016/j.ejbt.2020.06.006

29. Bodin H, Asp H, Hultberg M (2017) Effects of biopellets composed of microalgae and fungi on cadmium present at environmentally relevant levels in water. Int J Phytoremediation 19:500–4. https://doi.org/10.1080/15226514.2016.1244170

30. Oh JJ, Kim JY, Kim YJ. *et al.* (2021) Utilization of extracellular fungal melanin as an eco-friendly biosorbent for treatment of metal-contaminated effluents. Chemosphere 272:129884. https://doi.org/10.1016/j.chemosphere.2021.129884

31. Ganesh Kumar A, Manisha D, Sujitha K. *et al*. (2021) Genome sequence analysis of deep sea Aspergillus sydowii BOBA1 and effect of high pressure on biodegradation of spent engine oil. Sci Rep 11:1–19. https://doi.org/10.1038/s41598-021-88525-9

32. Sánchez-Vázquez V, Shirai K, González I. *et al*. (2018) Polycyclic aromatic hydrocarbon-emulsifier protein produced by Aspergillus brasiliensis (niger) in an airlift bioreactor following an electrochemical pretreatment. Bioresour Technol 256:408–13. https://doi.org/10.1016/j.biortech.2018.02.043

33. Barnes NM, Khodse VB, Lotlikar NP. *et al.* (2018) Bioremediation potential of hydrocarbon-utilizing fungi from select marine niches of India. 3 Biotech 8:21. https://doi.org/10.1007/s13205-017-1043-8

34. Kumar V, Dwivedi SK (2021) Bioremediation mechanism and potential of copper by actively growing fungus Trichoderma lixii CR700 isolated from electroplating wastewater. J Environ Manage 277:111370. https://doi.org/10.1016/j.jenvman.2020.111370

35. Wulandari R, Lotrakul P, Punnapayak H. *et al.* (2021) Toxicity evaluation and biodegradation of phenanthrene by laccase from Trametes polyzona PBURU 12. 3 Biotech 11:1–11. https://doi.org/10.1007/s13205-020-02556-z

36. Liu H, Yuan M, Tan S. *et al*. (2015) Enhancement of arbuscular mycorrhizal fungus (Glomus versiforme) on the growth and Cd uptake by Cd-hyperaccumulator Solanum nigrum. Appl Soil Ecol 89:44–9. https://doi.org/10.1016/j.apsoil.2015.01.006

37. Wang G, Wang L, Ma F (2022) Effects of earthworms and arbuscular mycorrhizal fungi on improvement of fertility and microbial communities of soils heavily polluted by cadmium. Chemosphere 286:131567. https://doi.org/10.1016/j.chemosphere.2021.131567

38. Birolli WG, de A. Santos D, Alvarenga N. *et al*. (2018) Biodegradation of anthracene and several PAHs by the marine-derived fungus Cladosporium sp. CBMAI 1237. Mar Pollut Bull 129:525–33. https://doi.org/10.1016/j.marpolbul.2017.10.023

39. Chaprão MJ, Ferreira INS, Correa PF. *et al* (2015) Application of bacterial and yeast biosurfactants for enhanced removal and biodegradation of motor oil from contaminated sand. Electron J Biotechnol 18:471–9. https://doi.org/10.1016/j.ejbt.2015.09.005

40. Li Q, Li J, Jiang L. *et al.* (2021) Diversity and structure of phenanthrene degrading bacterial communities associated with fungal bioremediation in petroleum contaminated soil. J Hazard Mater 403:123895. https://doi.org/10.1016/j.jhazmat.2020.123895

41. Medaura MC, Guivernau M, Moreno-Ventas X. *et al.* (2021) Bioaugmentation of Native Fungi, an Efficient Strategy for the Bioremediation of an Aged Industrially Polluted Soil With Heavy Hydrocarbons. Front Microbiol 12:1–18. https://doi.org/10.3389/fmicb.2021.626436

42. Li X, Lan X, Feng X. *et al.* (2021) Biosorption capacity of Mucor circinelloides bioaugmented with Solanum nigrum L. for the cleanup of lead, cadmium and arsenic. Ecotoxicol Environ Saf 212:112014. https://doi.org/10.1016/j.ecoenv.2021.112014

43. de Lima Souza HM, Sette LD, da Mota AJ. *et al* (2016) Filamentous Fungi Isolates of Contaminated Sediment in the Amazon Region with the Potential for Benzo(a)pyrene Degradation. Water, Air, Soil Pollut 227:431. https://doi.org/10.1007/s11270-016-3101-y

44. Lee AH, Lee H, Mok Y. *et al.*(2020) A proposed stepwise screening framework for the selection of polycyclic aromatic hydrocarbon (PAH)- degrading white rot fungi. Bioprocess Biosyst Eng 43:767–83. https://doi.org/10.1007/s00449-019-02272-w

45. da Silva AF, Banat IM, Giachini AJ. *et al*. (2021) Fungal biosurfactants, from nature to biotechnological product: bioprospection, production and potential applications. Bioprocess Biosyst Eng 44:2003–34. https://doi.org/10.1007/s00449-021-02597-5

46. Fasim A, More VS, More SS (2021) Large-scale production of enzymes for biotechnology uses. Curr Opin Biotechnol

69:68–76. https://doi.org/10.1016/j.copbio.2020.12.002

47. Feng L, Jiang X, Huang Y. *et al.* (2021) Petroleum hydrocarbon-contaminated soil bioremediation assisted by isolated bacterial consortium and sophorolipid. Environ Pollut 273:116476. https://doi.org/10.1016/j.envpol.2021.116476

48. Shah V, Daverey A (2021) Effects of sophorolipids augmentation on the plant growth and phytoremediation of heavy metal contaminated soil. J Clean Prod 280:124406. https://doi.org/10.1016/j.jclepro.2020.124406

49. Eibes G, Arca-Ramos A, Feijoo G. *et al.* (2015) Enzymatic technologies for remediation of hydrophobic organic pollutants in soil. Appl Microbiol Biotechnol 99:8815–29. https://doi.org/10.1007/s00253-015-6872-y

50. Rathankumar AK, Saikia K, Kumar PS. *et al*. (2021) Surfactant-aided mycoremediation of soil contaminated with polycyclic aromatic hydrocarbon (PAHs): progress , limitation , and countermeasures. J Chem Technol Biotechnol 391-408. https://doi.org/10.1002/jctb.6721

51. Mishra S, Lin Z, Pang S. *et al.* (2021) Biosurfactant is a powerful tool for the bioremediation of heavy metals from contaminated soils. J Hazard Mater 418:126253. https://doi.org/10.1016/j.jhazmat.2021.126253

52. Elshafie HS, Camele I, Sofo A. *et al* (2020) Mycoremediation effect of Trichoderma harzianum strain T22 combined with ozonation in diesel-contaminated sand. Chemosphere 252. https://doi.org/10.1016/j.chemosphere.2020.126597

53. Peidro-Guzmán H, Pérez-Llano Y, González-Abradelo D. *et al*. (2021) Transcriptomic analysis of polyaromatic hydrocarbon degradation by the halophilic fungus Aspergillus sydowii at hypersaline conditions. Environ Microbiol 23:3435–59. https://doi.org/10.1111/1462-2920.15166

54. Bao Q, Huang L, Xiu J. *et al.* (2021) Study on the treatment of oily sludge in oil fields with lipopeptide/sophorolipid complex bio-surfactant. Ecotoxicol Environ Saf 212:111964. https://doi.org/10.1016/j.ecoenv.2021.111964

55. Mishra A, Malik A (2014) Novel fungal consortium for bioremediation of metals and dyes from mixed waste stream. Bioresour Technol 171:217–26. https://doi.org/10.1016/j.biortech.2014.08.047

56. Marchand C, St-Arnaud M, Hogland W. *et al.* (2017) Petroleum biodegradation capacity of bacteria and fungi isolated from petroleum-contaminated soil. Int Biodeterior Biodegrad 116:48–57. https://doi.org/10.1016/j.ibiod.2016.09.030

57. Wilson SC, Jones KC (1993) Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): A review. Environ Pollut 81:229–49. https://doi.org/10.1016/0269-7491(93)90206-4

58. Ghosal D, Ghosh S, Dutta TK. *et al.* (2016) Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): A review. Front Microbiol 7. https://doi.org/10.3389/fmicb.2016.01369

59. Khatoon H, Rai JPN, Jillani A (2021) Role of fungi in bioremediation of contaminated soil. Fungi Bio-Prospects in Sustainable Agriculture, Environment and Nano-technology. Elsevier 121–56. https://doi.org/10.1016/B978-0-12-821925-6.00007-1

60. Kadri T, Rouissi T, Kaur BS. *et al.* (2017) Biodegradation of polycyclic aromatic hydrocarbons (PAHs) by fungal enzymes: A review. J Environ Sci 51:52–74. https://doi.org/10.1016/j.jes.2016.08.023

61. Chaillan F, Le Flèche A, Bury E. *et al*. (2004) Identification and biodegradation potential of tropical aerobic hydrocarbon-degrading microorganisms. Res Microbiol 155:587–95. doi.org/10.1016/j.jes.2016.08.023

62. Dallinger A, Duldhardt I, Kabisch J. *et al.* (2016) Biotransformation of cyclohexane and related alicyclic hydrocarbons by Candida maltosa and Trichosporon species. Int Biodeterior Biodegrad 107:132–9. https://doi.org/10.1016/j.ibiod.2015.11.015

63. Singh RK, Tripathi R, Ranjan A. *et al.* (2020) Fungi as potential candidates for bioremediation. Abatement of Environmental Pollutants 177–91. https://doi.org/10.1016/B978-0-12-818095-2.00009-6

64. Fayeulle A, Veignie E, Slomianny C. *et al.* (2014) Energy-dependent uptake of benzo [a] pyrene and its cytoskeleton-dependent intracellular transport by the telluric fungus Fusarium solani. Environ Sci Pollut Res 3515–23. https://doi.org/10.1007/s11356-013-2324-3

65. Delsarte I, Rafin C, Mrad F. *et al.* (2018) Lipid metabolism and benzo [a] pyrene degradation by Fusarium solani : an unexplored potential. Environ Sci Pollut Res 25:12177–82. https://doi.org/10.1007/s11356-017-1164-y

66. Chen W, Lee MK, Jefcoate C. *et al.* (2014) Fungal cytochrome P450 monooxygenases: Their distribution, structure, functions, family expansion, and evolutionary origin. Genome Biol Evol 6:1620–34. https://doi.org/10.1093/gbe/evu132

67. Hadibarata T, Tachibana S, Itoh K (2009) Biodegradation of chrysene, an aromatic hydrocarbon by Polyporus sp. S133 in liquid medium. J Hazard Mater 164:911–7. https://doi.org/https://doi.org/10.1016/j.jhazmat.2008.08.081

68. Ostrem Loss EM, Yu JH (2018) Bioremediation and microbial metabolism of benzo(a)pyrene. Mol Microbiol 109:433–44. https://doi.org/10.1111/mmi.14062

69. Park H, Min B, Jang Y. *et al*. (2019) Comprehensive genomic and transcriptomic analysis of polycyclic aromatic hydrocarbon degradation by a mycoremediation fungus, Dentipellis sp. KUC8613. Appl Microbiol Biotechnol 103:8145–55. https://doi.org/10.1007/s00253-019-10089-6

70. Ba S, Kumar VV (2017) Recent developments in the use of tyrosinase and laccase in environmental applications. Crit Rev Biotechnol 37:819–32. https://doi.org/10.1080/07388551.2016.1261081

71. Roshandel F, Saadatmand S, Iranbakhsh A. *et al.* (2021) Mycoremediation of oil contaminant by Pleurotus florida (P . Kumm) in liquid culture. Fungal Biol 125:667–78. https://doi.org/10.1016/j.funbio.2021.04.002

72. Catherine H, Penninckx M, Frédéric D (2016) Product formation from phenolic compounds removal by laccases: A review. Environ Technol Innov 5:250–66. https://doi.org/https://doi.org/10.1016/j.eti.2016.04.001

73. Mohammadi M, As'habi MA, Salehi P. *et al.* (2018) Immobilization of laccase on epoxy-functionalized silica and its application in biodegradation of phenolic compounds. Int J Biol Macromol 109:443–7. https://doi.org/10.1016/j.ijbiomac.2017.12.102

74. Kües U (2015) Fungal enzymes for environmental management. Curr Opin Biotechnol 33:268–78. https://doi.org/10.1016/j.copbio.2015.03.006

75. Vipotnik Z, Michelin M, Tavares T (2022) Biodegradation of chrysene and benzo [a] pyrene and removal of metals from naturally contaminated soil by isolated Trametes versicolor strain and laccase produced thereof. Environ Technol Innov 28:102737. https://doi.org/10.1016/j.eti.2022.102737

76. Zeng J, Li Y, Dai Y. *et al.* (2021) Effects of polycyclic aromatic hydrocarbon structure on PAH mineralization and toxicity to soil microorganisms after oxidative bioremediation by laccase. Environ Pollut 287:117581. https://doi.org/10.1016/j.envpol.2021.117581

77. Zeng J, Zhu Q, Wu Y. *et al.* (2018) Oxidation of benzo[a]pyrene by laccase in soil enhances bound residue formation and reduces disturbance to soil bacterial community composition. Environ Pollut 242:462–9. https://doi.org/10.1016/j.envpol.2018.06.075

78. Wu Y, Teng Y, Li Z. *et al.* (2008) Potential role of polycyclic aromatic hydrocarbons (PAHs) oxidation by fungal laccase in the remediation of an aged contaminated soil. Soil Biol Biochem 40:789–96. https://doi.org/10.1016/j.soilbio.2007.10.013

79. Vipotnik Z, Michelin M, Tavares T (2022) Rehabilitation of a historically contaminated soil by different laccases and laccase-mediator system. J Soils Sediments 22:1546–54. https://doi.org/10.1007/s11368-021-03125-4

80. Li X, Lin X, Yin R. *et al*. (2010) Optimization of laccase-mediated benzo[a]pyrene oxidation and the bioremedial application in aged polycyclic aromatic hydrocarbons-contaminated soil. J Heal Sci 56:534–40. https://doi.org/10.1248/jhs.56.534

81. Mate DM, Alcalde M (2015) Laccase engineering: From rational design to directed evolution. Biotechnol Adv 33:25–40. https://doi.org/https://doi.org/10.1016/j.biotechadv.2014.12.007

82. Deng J, Wang H, Zhan H. *et al*. (2022) Catalyzed degradation of polycyclic aromatic hydrocarbons by recoverable magnetic chitosan immobilized laccase from Trametes versicolor. Chemosphere 301:134753. https://doi.org/10.1016/j.chemosphere.2022.134753

83. Cañas AI, Camarero S (2010) Laccases and their natural mediators: Biotechnological tools for sustainable eco-friendly processes. Biotechnol Adv 28:694–705. https://doi.org/10.1016/j.biotechadv.2010.05.002

84. Wu Y, Jiang Y, Jiao J. *et al*. (2014) Adsorption of Trametes versicolor laccase to soil iron and aluminum minerals: Enzyme activity, kinetics and stability studies. Colloids Surfaces B Biointerfaces 114:342–8. https://doi.org/10.1016/j.colsurfb.2013.10.016

85. Yang Z, Mao X, Cui J. *et al*. (2021) Enhancement and analysis of Anthracene degradation by Tween 80 in LMS-HOBt. Sci Rep 11:1–11. https://doi.org/10.1038/s41598-021-90609-5

86. Kästner M, Nowak KM, Miltner A. *et al.* (2014) Classification and Modelling of Nonextractable Residue (NER) Formation of Xenobiotics in Soil – A Synthesis. Crit Rev Environ Sci Technol 44:2107–71. https://doi.org/10.1080/10643389.2013.828270

87. Li X, Lin X, Zhang J. *et al*. (2010) Degradation of polycyclic aromatic hydrocarbons by crude extracts from spent mushroom substrate and its possible mechanisms. Curr Microbiol 60:336–42. https://doi.org/10.1007/s00284-009-9546-0

88. Chiadò A, Bosco F, Bardelli M. et al (2021) Rational engineering of the lccβ T. versicolor laccase for the mediator-less oxidation of large polycyclic aromatic hydrocarbons. Comput Struct Biotechnol J 19:2213–22. https://doi.org/10.1016/j.csbj.2021.03.017

89. Alcalde M (2015) Engineering the ligninolytic enzyme consortium. Trends Biotechnol 33:155–62. https://doi.org/10.1016/j.tibtech.2014.12.007

90. Chen X, Hu Z, Xie H. *et al.* (2021) Enhanced biocatalysis of phenanthrene in aqueous phase by novel CA-Ca-SBE-laccase biocatalyst: Performance and mechanism. Colloids Surfaces A Physicochem Eng Asp 611:125884. https://doi.org/10.1016/j.colsurfa.2020.125884

91. Zhang Q, Zeng G, Chen G. *et al*. (2015) The Effect of Heavy Metal-Induced Oxidative Stress on the Enzymes in White Rot Fungus Phanerochaete chrysosporium. Appl Biochem Biotechnol 175:1281–93. https://doi.org/10.1007/s12010-014-1298-z

92. Mota EA, Felestrino ÉB, Leão VA. *et al.* (2020) Manganese (II) removal from aqueous solutions by Cladosporium halotolerans and Hypocrea jecorina. Biotechnol Reports 25:e00431. https://doi.org/10.1016/j.btre.2020.e00431

93. Chakraborty S, Mukherjee A, Das TK (2013) Biochemical characterization of a lead-tolerant strain of Aspergillus foetidus: Animplication of bioremediation of lead from liquid media. Int Biodeterior Biodegrad 84:134–42. https://doi.org/10.1016/j.ibiod.2012.05.031

94. Mosa KA, Saadoun I, Kumar K. *et al*. (2016) Potential biotechnological strategies for the cleanup of heavy metals and metalloids. Front Plant Sci 7:1–14. https://doi.org/10.3389/fpls.2016.00303

95. Fernández PM, Viñarta SC, Bernal AR. *et al.* (2018) Bioremediation strategies for chromium removal: Current research, scale-up approach and future perspectives. Chemosphere 208:139–48. https://doi.org/10.1016/j.chemosphere.2018.05.166

96. Alsharari SF, Tayel AA, Moussa SH (2018) Soil emendation with nano-fungal chitosan for heavy metals biosorption. Int J Biol Macromol 118:2265–8. https://doi.org/10.1016/j.ijbiomac.2018.07.103

97. Lu N, Hu T, Zhai Y. *et al.* (2020) Fungal cell with artificial metal container for heavy metals biosorption : Equilibrium , kinetics study and mechanisms analysis. Environ Res 182:109061. https://doi.org/10.1016/j.envres.2019.109061

98. Ma X, Cui W, Yang L. *et al.* (2015) Efficient biosorption of lead (II) and cadmium (II) ions from aqueous solutions by functionalized cell with intracellular CaCO3 mineral scaffolds. Bioresour Technol 185:70–8. https://doi.org/10.1016/j.biortech.2015.02.074

99. Dusengemungu L, Kasali G, Gwanama C (2020) Recent Advances in Biosorption of Copper and Cobalt by Filamentous Fungi. Front Microbiol 11:1–16. https://doi.org/10.3389/fmicb.2020.582016

100. Xu P, Leng Y, Zeng G. *et al*. (2015) Cadmium induced oxalic acid secretion and its role in metal uptake and detoxification mechanisms in Phanerochaete chrysosporium. Appl Microbiol Biotechnol 99:435–43. https://doi.org/10.1007/s00253-014-5986-y

101. Harms H, Schlosser D, Wick LY (2011) Untapped potential: Exploiting fungi in bioremediation of hazardous chemicals. Nat Rev Microbiol 9:177–92. https://doi.org/10.1038/nrmicro2519

102. Hassan SE, Hijri M, St-Arnaud M (2013) Effect of arbuscular mycorrhizal fungi on trace metal uptake by sunflower plants grown on cadmium contaminated soil. N Biotechnol 30:780–7. https://doi.org/10.1016/j.nbt.2013.07.002

103. Muñoz AJ, Ruiz E, Abriouel H. *et al*. (2012) Heavy metal tolerance of microorganisms isolated from wastewaters: Identification and evaluation of its potential for biosorption. Chem Eng J 210:325–32. https://doi.org/10.1016/j.cej.2012.09.007

104. Barea-Sepúlveda M, Espada-Bellido E, Ferreiro-González M. *et al.* (2022) Toxic elements and trace elements in Macrolepiota procera mushrooms from southern Spain and northern Morocco. J Food Compos Anal 108:104419. https://doi.org/10.1016/j.jfca.2022.104419

105. Ahmed E, Holmström SJM (2014) Siderophores in environmental research: Roles and applications. Microb Biotechnol 7:196–208. https://doi.org/10.1111/1751-7915.12117

106. Yang J, Wang Q, Luo Q. *et al.* (2009) Biosorption behavior of heavy metals in bioleaching process of MSWI fly ash by Aspergillus niger 46:294–9. https://doi.org/10.1016/j.bej.2009.05.022

107. Boriová K, Čerňanský S, Matúš P. *et al.* (2014) Bioaccumulation and biovolatilization of various elements using filamentous fungus Scopulariopsis brevicaulis. Lett Appl Microbiol 59:217–23. https://doi.org/10.1111/lam.12266

108. Li Q, Liu J, Gadd GM (2020) Fungal bioremediation of soil co-contaminated with petroleum hydrocarbons and toxic metals. Appl Microbiol Biotechnol 104:8999–9008. https://doi.org/10.1007/s00253-020-10854-y

109. Souza EC, Vessoni-Penna TC, Oliveira RPS (2014) Biosurfactant-enhanced hydrocarbon bioremediation :An overview. Int Biodeterior Biodegradation 89:88–94. https://doi.org/10.1016/j.ibiod.2014.01.007

110. Al-Hawash AB, Zhang X, Ma F (2019) Removal and biodegradation of different petroleum hydrocarbons using the filamentous fungus Aspergillus sp. RFC- 1. Microbiologyopen 8:e00619. https://doi.org/10.1002/mbo3.619

111. Kamyabi A, Nouri H, Moghimi H (2017) Synergistic Effect of Sarocladium sp. and Cryptococcus sp. Co-Culture on Crude Oil Biodegradation and Biosurfactant Production. Appl Biochem Biotechnol 182:324–34. https://doi.org/10.1007/s12010-016-2329-8

112. Sarubbo LA, Rocha RB, Luna JM. *et al.* (2015) Some aspects of heavy metals contamination remediation and role of biosurfactants. Chem Ecol 31:707–23. https://doi.org/10.1080/02757540.2015.1095293

113. Bami MS, Estabragh MAR, Ohadi M. *et al*. (2022) Biosurfactants aided bioremediation mechanisms: A mini-review. Soil Sediment Contam An Int J 1–17. https://doi.org/10.1080/15320383.2021.2016603

114. Chandran P, Das N (2012) Role of sophorolipid biosurfactant in degradation of diesel oil by Candida tropicalis. Bioremediat J 16:19–30. https://doi.org/10.1080/10889868.2011.628351

115. Singh SK, Singh MK, Verma H. *et al*. (2021) Biosurfactant producing microbes for clean-up of soil contaminants. Microbe Mediated Remediation of Environmental Contaminants 89–93. https://doi.org/10.1016/B978-0-12-821199-1.00009-2

116. Santos DKF, Resende AHM, de Almeida DG. *et al*. (2017) Candida lipolytica UCP0988 Biosurfactant: Potential as a

Bioremediation Agent and in Formulating a Commercial Related Product. Front Microbiol 8:1–11. https://doi.org/10.3389/fmicb.2017.00767

117. Mulligan CN, Yong RN, Gibbs BF (2001) An evaluation of technologies for the heavy metal remediation of dredged sediments. J Hazard Mater 85:145–63. https://doi.org/10.1016/S0304-3894(01)00226-6

118. Pino-Herrera DO, Pechaud Y, Huguenot D. *et al*. (2017) Removal mechanisms in aerobic slurry bioreactors for remediation of soils and sediments polluted with hydrophobic organic compounds: An overview. J Hazard Mater 339:427–49. https://doi.org/10.1016/j.jhazmat.2017.06.013

119. Gałązka A, Grządziel J, Gałązka R *et al.* (2020) Fungal Community, Metabolic Diversity, and Glomalin-Related Soil Proteins (GRSP) Content in Soil Contaminated With Crude Oil After Long-Term Natural Bioremediation. Front Microbiol. (2020);11. https://doi.org/10.3389/fmicb.2020.572314

120. Puglisi I, Faedda R, Sanzaro V. *et al.* (2012) Identification of differentially expressed genes in response to mercury I and II stress in Trichoderma harzianum. Gene 506:325–30. https://doi.org/10.1016/j.gene.2012.06.091

121. Arima K, Kakinuma A, Tamura G (1968) Surfactin, a crystalline peptidelipid surfactant produced by Bacillus subtilis: isolation, characterization and its inhibition of fibrin clot formation. Biochem Biophys Res Commun 31:488–94. https://doi.org/10.1016/0006-291X(68)90503-2

122. Rehman R, Ishtiaq M, Ali N. *et al.* (2021) Crude oil biodegradation potential of biosurfactant-producing Pseudomonas aeruginosa and Meyerozyma sp . J Hazard Mater 418:126276. https://doi.org/10.1016/j.jhazmat.2021.126276

123. Niu Y, Fan L, Gu D. *et al.* (2017) Characterization, enhancement and modelling of mannosylerythritol lipid production by fungal endophyte Ceriporia lacerate CHZJU. Food Chem 228:610–7. https://doi.org/10.1016/j.foodchem.2017.02.042

124. Wang M, Mao W, Wang X. *et al*. (2019) Efficient simultaneous production of extracellular polyol esters of fatty acids and intracellular lipids from inulin by a deep-sea yeast Rhodotorula paludigena P4R5. Microb Cell Fact 18:149. https://doi.org/10.1186/s12934-019-1200-3

125. Chen S, Zhou Z, Tsang DCW. *et al*. (2020) Glomalin-related soil protein reduces the sorption of polycyclic aromatic hydrocarbons by soils. Chemosphere. (2020);260:127603. https://doi.org/https://doi.org/10.1016/j.chemosphere.2020.127603

126. Yuan B, Li H, Hong H. *et al.* (2022) Immobilization of lead (II) and zinc (II) onto glomalin-related soil protein (GRSP): Adsorption properties and interaction mechanisms. Ecotoxicol Environ Saf 236:113489. https://doi.org/10.1016/j.ecoenv.2022.113489

127. Zadeh PH, Moghimi H, Hamedi J (2018) Biosurfactant production by Mucor circinelloides : Environmental applications and surface-active properties. Eng Life Sci 18:317–25. https://doi.org/10.1002/elsc.201700149

128. Kreling NE, Zaparoli M, Margarites AC. *et al*. (2020) Extracellular biosurfactants from yeast and soil–biodiesel interactions during bioremediation. Int J Environ Sci Technol 17:395–408. https://doi.org/10.1007/s13762-019-02462-9

129. Kreling NE, Simon V, Fagundes VD. *et al.* (2020) Simultaneous Production of Lipases and Biosurfactants in Solid-State Fermentation and Use in Bioremediation. J Environ Eng 146:04020105. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001785

130. Goswami T, Tack FMG, McGachy L. *et al*. (2020) Remediation of Aviation Kerosene-Contaminated Soil by Sophorolipids from Candida bombicola CB 2107. Appl Sci 10:1–11. https://doi.org/10.3390/app10061981

131. Luna JM, Rufino RD, Sarubbo LA (2016) Biosurfactant from Candida sphaerica UCP0995 exhibiting heavy metal remediation properties. Process Saf Environ Prot 102:558–66. https://doi.org/10.1016/j.psep.2016.05.010

132. Arab F, Mulligan CN (2018) An eco-friendly method for heavy metal removal from mine tailings. Environ Sci Pollut Res 25:16202–16. https://doi.org/10.1007/s11356-018-1770-3

133. Qi X, Xu X, Zhong C. *et al.* (2018) Removal of Cadmium and Lead from Contaminated Soils Using Sophorolipids from Fermentation Culture of Starmerella bombicola CGMCC 1576 Fermentation. Int J Environ Res Public Health 15:2334. https://doi:10.3390/ijerph15112334

134. Batista RM, Rufino RD, Luna JM. *et al*. (2010) Effect of Medium Components on the Production of a Biosurfactant from Candida tropicalis Applied to the Removal of Hydrophobic Contaminants in Soil. Water Environ Res 82:418–25. https://doi.org/10.2175/106143009X12487095237279

135. Pele MA, Ribeaux DR, Vieira ER. *et al.* (2019) Conversion of renewable substrates for biosurfactant production by Rhizopus arrhizus UCP 1607 and enhancing the removal of diesel oil from marine soil. Electron J Biotechnol 38:40–8. https://doi.org/10.1016/j.ejbt.2018.12.003

136. Sobrinho HBS, Rufino RD, Luna JM. *et al*. (2008) Utilization of two agroindustrial by-products for the production of a surfactant by Candida sphaerica UCP0995. Process Biochem 43:912–7. https://doi.org/10.1016/j.procbio.2008.04.013

137. Olaniran A, Balgobind A, Pillay B (2013) Bioavailability of Heavy Metals in Soil: Impact on Microbial Biodegradation of Organic Compounds and Possible Improvement Strategies. Int J Mol Sci 14:10197–228. https://doi.org/10.3390/ijms140510197

138. Becarelli S, Chicca I, La China S, Siracusa G, Bardi A, Gullo M, et al (2021) A New Ciboria sp. for Soil

Mycoremediation and the Bacterial Contribution to the Depletion of Total Petroleum Hydrocarbons. Front Microbiol 12. https://doi.org/10.3389/fmicb.2021.647373

139. Al-Hawash AB, Alkooranee JT, Abbood HA. *et al* (2018) Isolation and characterization of two crude oil-degrading fungi strains from Rumaila oil field, Iraq. Biotechnol Reports 17:104–9. https://doi.org/10.1016/j.btre.2017.12.006

140. Fernández PM, Martorell MM, Blaser MG. *et al.* (2017) Phenol degradation and heavy metal tolerance of Antarctic yeasts. Extremophiles 21:445–57. https://doi.org/10.1007/s00792-017-0915-5

141. Shetaia YMH, El Khalik WAA, Mohamed TM. *et al.* (2016) Potential biodegradation of crude petroleum oil by newly isolated halotolerant microbial strains from polluted Red Sea area. Mar Pollut Bull 111:435–42. https://doi.org/10.1016/j.marpolbul.2016.02.035

142. Bik HM, Halanych KM, Sharma J. *et al.* (2012) Dramatic shifts in benthic microbial eukaryote communities following the deepwater horizon oil spill. PLoS One 7. https://doi.org/10.1371/journal.pone.0038550

143. Chang J, Duan Y, Dong J. *et al* (2019) Bioremediation of Hg-contaminated soil by combining a novel Hg-volatilizing Lecythophora sp. fungus, DC-F1, with biochar: Performance and the response of soil fungal community. Sci Total Environ 671:676–84. https://doi.org/10.1016/j.scitotenv.2019.03.409

144. Chaudhary P, Beniwal V, Sharma P. *et al.* (2022) Unloading of hazardous Cr and Tannic Acid from real and synthetic waste water by novel fungal consortia. Environ Technol Innov 26:102230. https://doi.org/10.1016/j.eti.2021.102230

145. Williams W, Trindade M (2017) Metagenomics for the Discovery of Novel Biosurfactants. Functional Metagenomics: Tools and Applications. Cham: Springer International Publishing 95–117. https://doi.org/10.1007/978-3-319-61510-3_6

146. Prenafeta-Boldú FX, Kuhn A, Luykx DMAM. *et al.* (2001) Isolation and characterisation of fungi growing on volatile aromatic hydrocarbons as their sole carbon and energy source. Mycol Res 105:477–84. https://doi.org/10.1017/S0953756201003719

147. Satow MM, Attili-Angelis D, de Hoog GS. *et al.* (2008) Selective factors involved in oil flotation isolation of black yeasts from the environment. Stud Mycol 61:157–63. https://doi.org/10.3114/sim.2008.61.16

148. Poyntner C, Prem M, Mann O. *et al.* (2017) Selective screening: Isolation of fungal strains from contaminated soils in Austria. Bodenkultur 68:157–69. https://doi.org/10.1515/boku-2017-0014

149. Lee H, Jang Y, Choi Y-S. *et al*. (2014) Biotechnological procedures to select white rot fungi for the degradation of PAHs. J Microbiol Methods 97:56–62. https://doi.org/https://doi.org/10.1016/j.mimet.2013.12.007

150. Ortega-González DK, Cristiani-Urbina E, Flores-Ortíz CM. *et al.* (2014) Evaluation of the Removal of Pyrene and Fluoranthene by Ochrobactrum anthropi, Fusarium sp. and Their Coculture. Appl Biochem Biotechnol 175:1123–38. https://doi.org/10.1007/s12010-014-1336-x

151. Bovio E, Gnavi G, Prigione V. *et al*. (2017) The culturable mycobiota of a Mediterranean marine site after an oil spill: Isolation, identification and potential application in bioremediation. Sci Total Environ 576:310–8. https://doi.org/10.1016/j.scitotenv.2016.10.064

152. Ameen F, Moslem M, Hadi S. *et al*. (2016) Biodegradation of diesel fuel hydrocarbons by mangrove fungi from Red Sea Coast of Saudi Arabia. Saudi J Biol Sci 23:211–8. https://doi.org/10.1016/j.sjbs.2015.04.005

153. Atakpa EO, Zhou H, Jiang L. *et al*. (2022) Improved degradation of petroleum hydrocarbons by co-culture of fungi and biosurfactant-producing bacteria. Chemosphere 290:133337. https://doi.org/10.1016/j.chemosphere.2021.133337

154. Ghorbannezhad H, Moghimi H, Mohammad S. *et al.* (2021) Evaluation of pyrene and tetracosane degradation by mixed-cultures of fungi and bacteria. J Hazard Mater 416:126202. https://doi.org/10.1016/j.jhazmat.2021.126202

155. Khan I, Ali M, Aftab M. *et al* (2019) Mycoremediation: a treatment for heavy metal-polluted soil using indigenous metallotolerant fungi. Environ Monit Assess 191. https://doi.org/10.1007/s10661-019-7781-9

156. Liu J, Yue P, Huang L. *et al.* (2020) Styrene removal with an acidic biofilter with four packing materials: Performance and fungal bioaerosol emissions. Environ Res 191:110154. https://doi.org/10.1016/j.envres.2020.110154

157. Fazli MM, Soleimani N, Mehrasbi M. *et al.* (2015) Highly cadmium tolerant fungi: Their tolerance and removal potential. J Environ Heal Sci Eng 13:1–9. https://doi.org/10.1186/s40201-015-0176-0

158. Reyes-César A, Absalón ÁE, Fernández FJ. *et al.* (2014) Biodegradation of a mixture of PAHs by non-ligninolytic fungal strains isolated from crude oil-contaminated soil. World J Microbiol Biotechnol 30:999–1009. https://doi.org/10.1007/s11274-013-1518-7

159. Grace Liu P-W, Chang TC, Whang L-M. *et al.* (2011) Bioremediation of petroleum hydrocarbon contaminated soil: Effects of strategies and microbial community shift. Int Biodeterior Biodegradation 65:1119–27. https://doi.org/10.1016/j.ibiod.2011.09.002

160. Zafra G, Absalón ÁE, Anducho-Reyes MÁ. *et al.* (2017) Construction of PAH-degrading mixed microbial consortia by induced selection in soil. Chemosphere 172:120–6. https://doi.org/10.1016/j.chemosphere.2016.12.038

161. Hesham AEL, Khan S, Tao Y. *et al.* (2012) Biodegradation of high molecular weight PAHs using isolated yeast mixtures: Application of meta-genomic methods for community structure analyses. Environ Sci Pollut Res 19:3568–78. https://doi.org/10.1007/s11356-012-0919-8

162. Gorfer M, Persak H, Berger H. *et al.* (2009) Identification of heavy metal regulated genes from the root associated ascomycete Cadophora finlandica using a genomic microarray. Mycol Res 113:1377–88. https://doi.org/10.1016/j.mycres.2009.09.005

163. Trippe KM, Wolpert TJ, Hyman MR. *et al.* (2014) RNAi silencing of a cytochrome P450 monoxygenase disrupts the ability of a filamentous fungus, Graphium sp., to grow on short-chain gaseous alkanes and ethers. Biodegradation 25:137–51. https://doi.org/10.1007/s10532-013-9646-1

164. De Oliveira VH, Ullah I, Dunwell JM. *et al.* (2020) Bioremediation potential of Cd by transgenic yeast expressing a metallothionein gene from Populus trichocarpa. Ecotoxicol Environ Saf 202:110917. https://doi.org/10.1016/j.ecoenv.2020.110917

165. Mäkelä MR, Tuomela M, Hatakka A. *et al.* (2020) Fungal Laccases and Their Potential in Bioremediation Applications. Laccases in Bioremediation and Waste Valorisation 1–25. https://doi.org/10.1007/978-3-030-47906-0_1

166. Téllez-Jurado A, Arana-Cuenca A, Becerra AEG. *et al.* (2006) Expression of a heterologous laccase by Aspergillus niger cultured by solid-state and submerged fermentations. Enzyme Microb Technol 38:665–9. https://doi.org/10.1016/j.enzmictec.2005.07.021

167. Balcázar-López E, Méndez-Lorenzo LH, Batista-García RA. *et al*. (2016) Xenobiotic Compounds Degradation by Heterologous Expression of a Trametes sanguineus Laccase in Trichoderma atroviride. PLoS One 11:e0147997. https://doi.org/10.1371/journal.pone.0147997

168. Nikolaivits E, Dimarogona M, Karagiannaki I. *et al.* (2018) Versatile fungal polyphenol oxidase with chlorophenol bioremediation potential: Characterization and protein engineering. Appl Environ Microbiol 84:1–16. https://doi.org/10.1128/AEM.01628-18

169. Syed K, Porollo A, Lam YW. *et al.* (2013) CYP63A2, a catalytically versatile fungal P450 monooxygenase capable of oxidizing higher-molecular-weight polycyclic aromatic hydrocarbons, alkylphenols, and alkanes. Appl Environ Microbiol 79:2692–702. https://doi.org/10.1128/AEM.03767-12

170. Boczonádi I, Török Z, Jakab Á. *et al*. (2020) Increased Cd2+ biosorption capability of Aspergillus nidulans elicited by crpA deletion. J Basic Microbiol 60:574–84. https://doi.org/10.1002/jobm.202000112

171. Oliveira JS, Araújo W, Lopes Sales AI. *et al*. (2015) BioSurfDB: knowledge and algorithms to support biosurfactants and biodegradation studies. Database 2015:1–8. https://doi.org/10.1093/database/bav033

172. Dhanjal DS, Sharma D (2018) Microbial Metagenomics for Industrial and Environmental Bioprospecting: The Unknown Envoy. Microbial Bioprospecting for Sustainable Development. Springer Singapore 327–52. https://doi.org/10.1007/978-981-13-0053-0_18

173. Sun Y, Liu L, Zeng J.*et al.* (2020). Enhanced cometabolism of benzo (a) anthracene by the lignin monomer vanillate is related to structural and functional responses of the soil microbiome. Soil Biol Biochem 149:107908. https://doi.org/10.1016/j.soilbio.2020.107908

174. Kolhe N, Kulkarni A, Zinjarde S. *et al.* (2021) Transcriptome Response of the Tropical Marine Yeast Yarrowia lipolytica on Exposure to Uranium. Curr Microbiol 78:2033–43. https://doi.org/10.1007/s00284-021-02459-z

175. Li J, Xu Y, Song Q. *et al.* (2021) Transmembrane transport mechanism of n-hexadecane by Candida tropicalis: Kinetic study and proteomic analysis. Ecotoxicol Environ Saf 209:111789 https://doi.org/10.1016/j.ecoenv.2020.111789

176. Rissoni Toledo GA, Reyes Andrade JC, Palmieri MC. *et al.* (2021) Innovative method for encapsulating highly pigmented biomass from Aspergillus nidulans mutant for copper ions removal and recovery. PLoS One 16:1–21. https://doi.org/10.1371/journal.pone.0259315

177. Han J, Hu L, He L. *et al.* (2020) Preparation and uranium (VI) biosorption for tri-amidoxime modified marine fungus material. Environ Sci Pollut Res 27:37313–23. https://doi.org/10.1007/s11356-020-07746-z

178. Robichaud K, Girard C, Dagher D. *et al.* (2019) Local fungi, willow and municipal compost effectively remediate petroleum-contaminated soil in the Canadian North. Chemosphere 220:47–55. https://doi.org/10.1016/j.chemosphere.2018.12.108

179. Ariste AF, Batista-García RA, Vaidyanathan VK. *et al.* (2020) Mycoremediation of phenols and polycyclic aromatic hydrocarbons from a biorefinery wastewater and concomitant production of lignin modifying enzymes. J Clean Prod 253. https://doi.org/10.1016/j.jclepro.2019.119810

180. Covino S, D'Annibale A, Stazi SR. *et al.* (2015) Assessment of degradation potential of aliphatic hydrocarbons by autochthonous filamentous fungi from a historically polluted clay soil. Sci Total Environ 505:545–54. https://doi.org/10.1016/j.scitotenv.2014.10.027

181. Fan M-Y, Xie R-J, Qin G (2014) Bioremediation of petroleum-contaminated soil by a combined system of biostimulation–bioaugmentation with yeast. Environ Technol 35:391–9. https://doi.org/10.1080/09593330.2013.829504

182. Potin O, Rafin C, Veignie E (2004) Bioremediation of an aged polycyclic aromatic hydrocarbons (PAHs)-contaminated soil by filamentous fungi isolated from the soil. Int Biodeterior Biodegradation 54:45–52. https://doi.org/10.1016/j.ibiod.2004.01.003

183. Valentín L, Lu-Chau TA, López C. *et al.* (2007) Biodegradation of dibenzothiophene, fluoranthene, pyrene and chrysene

in a soil slurry reactor by the white-rot fungus Bjerkandera sp. BOS55. Process Biochem 42:641–8. https://doi.org/10.1016/j.procbio.2006.11.011

184. Bilen Ozyurek S, Avcioglu NH, Seyis Bilkay I (2021) Mycoremediation potential of Aspergillus ochraceus NRRL 3174. Arch Microbiol 203:5937–50. https://doi.org/10.1007/s00203-021-02490-5

185. Rusinova-Videva S, Nachkova S, Adamov A. *et al.* (2020) Antarctic yeast Cryptococcus laurentii (AL65): biomass and exopolysaccharide production and biosorption of metals. J Chem Technol Biotechnol 95:1372–9. https://doi.org/10.1002/jctb.6321

186. Azin E, Moghimi H, Heidarytabar R (2018) Petroleum Degradation, Biosurfactant and Laccase Production by Fusarium neocosmosporiellum RH-10: A Microcosm Study. Soil Sediment Contam An Int J 27:329–42. https://doi.org/10.1080/15320383.2018.1473334

187. Hashem M, Alamri SA, Al-zomyh SSAA. *et al.* (2018) Biodegradation and detoxification of aliphatic and aromatic hydrocarbons by new yeast strains. Ecotoxicol Environ Saf 151:28–34. https://doi.org/10.1016/j.ecoenv.2017.12.064

188. Batista-García A, Vinoth V, Ariste A. *et al.* (2017) Simple screening protocol for identi fi cation of potential mycoremediation tools for the elimination of polycyclic aromatic hydrocarbons and phenols from hyperalkalophile industrial effluents. J Environ Manage 198:1–11. https://doi.org/10.1016/j.jenvman.2017.05.010

189. Moghimi H, Heidary Tabar R, Hamedi J (2017) Assessing the biodegradation of polycyclic aromatic hydrocarbons and laccase production by new fungus Trematophoma sp. UTMC 5003. World J Microbiol Biotechnol 33:136. https://doi.org/10.1007/s11274-017-2304-8

190. Ma X, Li X, Liu J. *et al.* (2021) Soil microbial community succession and interactions during combined plant / white-rot fungus remediation of polycyclic aromatic hydrocarbons. Sci Total Environ 752:142224. https://doi.org/10.1016/j.scitotenv.2020.142224

191. Alpat Ş, Alpat SK, Çadirci BH. *et al.* (2010) Effects of biosorption parameter: Kinetics, isotherm and thermodynamics for Ni(II) biosorption from aqueous solution by Circinella sp. Electron J Biotechnol 13. http://doi.org/10.2225/vol13-issue5-fulltext-20

192. El-Morsy ESM (2004) Cunninghamella echinulata a new biosorbent of metal ions from polluted water in Egypt. Mycologia 96:1183–9. https://doi.org/10.2307/3762133

193. Sharma R, Talukdar D, Bhardwaj S. *et al.* (2020) Bioremediation potential of novel fungal species isolated from wastewater for the removal of lead from liquid medium. Environ Technol Innov 18:100757. https://doi.org/10.1016/j.eti.2020.100757

194. Purchase D, Scholes LNL, Revitt DM. *et al.* (2009) Effects of temperature on metal tolerance and the accumulation of Zn and Pb by metal-tolerant fungi isolated from urban runoff treatment wetlands. J Appl Microbiol 106:1163–74. https://doi.org/10.1111/j.1365-2672.2008.04082.x

195. Xu F, Chen P, Li H. *et al.* (2021) Comparative transcriptome analysis reveals the differential response to cadmium stress of two Pleurotus fungi: Pleurotus cornucopiae and Pleurotus ostreatus. J Hazard Mater 416:125814. https://doi.org/https://doi.org/10.1016/j.jhazmat.2021.125814

196. Morales-Barrera L, Cristiani-Urbina E (2006) Removal of hexavalent chromium by Trichoderma viride in an airlift bioreactor. Enzyme Microb Technol 40:107–13. https://doi.org/10.1016/j.enzmictec.2005.10.044

197. Zhang Y, Liu J, Qin Y. *et al.* (2019) Performance and microbial community evolution of toluene degradation using a fungi-based bio-trickling filter. J Hazard Mater 365:642–9. https://doi.org/10.1016/j.jhazmat.2018.11.062

198. Vergara-Fernández A, Revah S, Moreno-Casas P. *et al.* (2018) Biofiltration of volatile organic compounds using fungi and its conceptual and mathematical modeling. Biotechnol Adv 36:1079–93. https://doi.org/10.1016/j.biotechadv.2018.03.008

199. Srivastava AK, Singh RK, Singh D (2021) Microbe-based bioreactor system for bioremediation of organic contaminants : present and future perspective 241–53. https://doi.org/10.1016/B978-0-12-821199-1.00020-1

200. Bruneel J, Huepe Follert JL, Laforce B. *et al.* (2020) Dynamic performance of a fungal biofilter packed with perlite for the abatement of hexane polluted gas streams using SIFT-MS and packing characterization with advanced X-ray spectroscopy. Chemosphere 253:1–10. https://doi.org/10.1016/j.chemosphere.2020.126684

201. Syed K, Doddapaneni H, Subramanian V. *et al.* (2010) Genome-to-function characterization of novel fungal P450 monooxygenases oxidizing polycyclic aromatic hydrocarbons (PAHs). Biochem Biophys Res Commun 399:492–7. https://doi.org/10.1016/j.bbrc.2010.07.094

202. Gu D, Xiang X, Wu Y. *et al.* (2022) Synergy between fungi and bacteria promotes polycyclic aromatic hydrocarbon cometabolism in lignin-amended soil. J Hazard Mater 425:127958. https://doi.org/10.1016/j.jhazmat.2021.127958

203. Al-Hawash AB, Dragh MA, Li S. *et al.* (2018) Principles of microbial degradation of petroleum hydrocarbons in the environment. Egypt J Aquat Res 44:71–6. https://doi.org/10.1016/j.ejar.2018.06.001

204. Zhang X, Kong D, Liu X. *et al.* (2021) Combined microbial degradation of crude oil under alkaline conditions by Acinetobacter baumannii and Talaromyces sp. Chemosphere 273:129666. https://doi.org/10.1016/j.chemosphere.2021.129666

205. da Rocha Junior RB, Meira HM, Almeida DG. *et al.* (2019) Application of a low-cost biosurfactant in heavy metal remediation processes. Biodegradation 30:215–33. https://doi.org/10.1007/s10532-018-9833-1

206. Rufino RD, Luna JM, Marinho PHC. *et al.* (2013) Removal of petroleum derivative adsorbed to soil by biosurfactant Rufisan produced by Candida lipolytica. J Pet Sci Eng 109:117–22. https://doi.org/10.1016/j.petrol.2013.08.014

207. Thomsen CN, Hart MM (2018) Using invasion theory to predict the fate of arbuscular mycorrhizal fungal inoculants. Biol Invasions 20:2695–706. https://doi.org/10.1007/s10530-018-1746-8

208. Chaudhary T, Dixit M, Gera R. *et al.* (2020) Techniques for improving formulations of bioinoculants. 3 Biotech 10:199. https://doi.org/10.1007/s13205-020-02182-9

209. Chandran P, Das N (2011) Degradation of diesel oil by immobilized Candida tropicalis and biofilm formed on gravels. Biodegradation 22:1181–9. https://doi.org/10.1007/s10532-011-9473-1

210. Nowak K, Wiater A, Choma A. *et al.* (2019) Fungal (1→3)-α-D-glucans as a new kind of biosorbent for heavy metals. Int J Biol Macromol 137:960–5. https://doi.org/10.1016/j.ijbiomac.2019.07.036

211. Cobas M, Ferreira L, Tavares T. *et al.* (2013) Development of permeable reactive biobarrier for the removal of PAHs by Trichoderma longibrachiatum. Chemosphere 91:711–6. https://doi.org/10.1016/j.chemosphere.2013.01.028

212. Dai Y, Yin L, Niu J (2011) Laccase-carrying electrospun fibrous membranes for adsorption and degradation of PAHs in shoal soils. Environ Sci Technol 45:10611–8. https://doi.org/10.1021/es203286e

213. Mukherjee T, Chakraborty S, Biswas AA. *et al.* (2017) Bioremediation potential of arsenic by non-enzymatically biofabricated silver nanoparticles adhered to the mesoporous carbonized fungal cell surface of Aspergillus foetidus MTCC8876. J Environ Manage 201:435–46. https://doi.org/10.1016/j.jenvman.2017.06.030

214. Tang J, He J, Xin X. *et al.* (2018) Biosurfactants enhanced heavy metals removal from sludge in the electrokinetic treatment. Chem Eng J 334:2579–92. https://doi.org/10.1016/j.cej.2017.12.010

215. Camargo FP, Prado PF do, Tonello PS. *et al.* (2018) Bioleaching of toxic metals from sewage sludge by co-inoculation of Acidithiobacillus and the biosurfactant-producing yeast Meyerozyma guilliermondii. J Environ Manage 211:28–35. https://doi.org/10.1016/j.jenvman.2018.01.045

216. Ye M, Sun M, Wan J. *et al.* (2016) Feasibility of lettuce cultivation in sophoroliplid-enhanced washed soil originally polluted with Cd, antibiotics, and antibiotic-resistant genes. Ecotoxicol Environ Saf 124:344–50. https://doi.org/10.1016/j.ecoenv.2015.11.013

217. Deng S, Zeng D (2017) Removal of phenanthrene in contaminated soil by combination of alfalfa , white-rot fungus, and earthworms. Environ Sci Pollut Res 24:7565–7571. https://doi.org/10.1007/s11356-017-8466-y

218. Li X, Chen AY, Wu Y. *et al.* (2018) Applying β-cyclodextrin to amaranth inoculated with white-rot fungus for more efficient remediation of soil co-contaminated with Cd and BDE-209. Sci Total Environ 634:417–26. https://doi.org/10.1016/j.scitotenv.2018.03.310

219. Shi Z, Liu J, Tang Z. *et al.* (2020) Vermiremediation of organically contaminated soils: Concepts, current status, and future perspectives. Appl Soil Ecol 147:103377. https://doi.org/10.1016/j.apsoil.2019.103377

220. Chachina SB, Voronkova NA, Baklanova ON (2015) Biological remediation of the engine lubricant oil-contaminated soil with three kinds of earthworms, Eisenia fetida, Eisenia andrei Dendrobena veneta, and a mixture of microorganisms. Procedia Eng 113:113–23. https://doi.org/10.1016/j.proeng.2015.07.302

221. Hassan A, Periathamby A, Ahmed A. *et al.* (2020) Effective bioremediation of heavy metal–contaminated landfill soil through bioaugmentation using consortia of fungi. J Soils Sediments 20:66–80. https://doi.org/10.1007/s11368-019-02394-4

222. Chandra S, Sharma R, Singh K. *et al.* (2013) Application of bioremediation technology in the environment contaminated with petroleum hydrocarbon. Ann Microbiol 63:417–31. https://doi.org/10.1007/s13213-012-0543-3

223. Ahmad I (2022) Microalgae–Bacteria Consortia: A Review on the Degradation of Polycyclic Aromatic Hydrocarbons (PAHs). Arab J Sci Eng 47:19–43. https://doi.org/10.1007/s13369-021-06236-9

224. Leng L, Li W, Chen J. *et al.* (2021) Co-culture of fungi-microalgae consortium for wastewater treatment: A review. Bioresour Technol 330:125008. https://doi.org/10.1016/j.biortech.2021.1

225. Al-Fawwaz AT, Jacob JH, Al-wahishe TE (2016) Bioremoval Capacity Of Phenol By Green Micro-Algal And Fungal Species Isolated From Dry Environment. Int J Sci Technol Res 5:155–60.