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Review

In silico bioremediation of polycyclic aromatic hydrocarbon: A frontier in environmental chemistry

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ABSTRACT

In recent years, the number of studies in the field of bioremediation has been growing steadily. Although a large number of studies provide information that is highly detailed and offer great amounts of knowledge on a given subject, the downside is that the hunt for more information requires the combined efforts of researchers from many areas, which are becoming increasingly difficult to attain. In this review, we present an overview of recent work investigating enzyme degradation of polycyclic aromatic hydrocarbons. In the first part, this review examines several of the new enzymes able to degrade pollutants, with special attention being given to those with a well-resolved structure. The second part explores some of the most recent work in which computational approaches, such as molecular dynamics, docking, density functional theory and database retrieval, have been employed to study enzymes with specific bioremediation activities.

1. Introduction

Bioremediation is the process by which living organisms, generally bacteria, degrade or transform hazardous compounds into less toxic compounds [1]. *At present, two classes of pollutants represent important challenges for bioremediation; one is polycyclic aromatic hydrocarbons (PAHs), and the second is halogenated hydrocarbons.* The management of PAHs is considered challenging for many reasons. Due to their hydrophobicity, these compounds tend to accumulate in soil organic matter; thus, their desorption from soil limits their availability to microorganisms for biodegradation [2]. PAHs are compounds with two or more fused benzene rings. They are formed during the incomplete thermal combustion of solid and liquid fuels or are derived from high-temperature (500–800 °C) industrial activities or from the injection of organic materials, including coal tars, crude oil and petroleum products [3], at temperatures below 300 °C. Moreover, nautical vessel effluents and spills produce serious aquatic pollution. As a class, PAHs are relatively unreactive chemically, with low solubility in water, high melting and boiling points and low vapour pressure [4]. They are ubiquitously present, toxic contaminants. PAHs are particularly good substrates for the cytochrome P-450 found in mammalian livers, where they are converted into epoxides that may bind to DNA [5]. These epoxides, particularly those derived from PAHs with exposed "bay regions" (e.g., chrysene), are highly potent xenobiotics and suspected [6,7] of being mutagenic and carcinogenic [8].

PAHs released into the environment could volatilise, photooxidise, chemically oxidise, bioaccumulate, or adsorb onto soil [9–11].

The bioremediation of these molecules is typically achieved by using bacteria to degrade them. Although this approach is very efficient and costs little, it is often limited by environmental conditions such as pH temperature and metal ions and salt may produce unwanted or toxic products. Despite the massive number of publications on bioremediation (more than 25,000 over the past 3 years, according to the ACS database), indicating great research interest worldwide, only a few (less than 1%) publications have focused on the chemical aspects thereof, using either a computational or multidisciplinary approach to study PAH. Given this broad background, it is difficult to focus attention on the very important aspect of enzymatic bioremediation because often the results are not straightforward. Thus, this work aims to highlight only those articles that play a key role in the search for enzymes, conditions or methods applicable to bioremediation.

This review is divided into two main chapters. The first addresses enzymes with a well-resolved 3D structure (X-ray or NMR data) that can degrade PAH, with some experimental details regarding new or lesser known enzymes provided; the second chapter addresses data and information on the computational aspects of enzymatic bioremediation.

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Table 1
Degrading activity of some simple bacteria, and in mixture [1,12].

Organism	PAH degradation (%), standard deviation it is ≤3%				
	Naphthalene	Acenaphthene	Fluorene	Anthracene	Pyrene
Pseudomonas sp.	15.5	28.0	24.4	25.4	92.3
Pycnoporus sanguineus	12.0	7.0	17.6	15.6	4.4
Coriolus versicolor	27.4	2.0	23.0	22.4	42.0
Pleurotus ostreatus	29.4	20.6	20.6	19.0	32.0
Fomitopsis palustris	19.5	7.5	7.0	31.7	7.3
Daedalea elegans	35.8	5.9	5.9	2.4	26.1
Pycnoporus sanguineus mixed with *Pseudomonas* sp.	13.5	29	24.2	11.4	17.4
Coriolus versicolor mixed with *Pseudomonas* sp.	15.5	27	24	25.0	93.7
Pleurotus ostreatus mixed with *Pseudomonas* sp.	13	25	19	20.0	17.0
Fomitopsis palustris mixed with *Pseudomonas* sp.	13.1	16.3	16.3	12.0	93.7
Daedalea elegan mixed with *Pseudomonas* sp.	23	14.9	14.9	3.4	46.4
Aerated soil at 40% WHC in presence of *Sphingomonas* and *Azospirillum*		100	100	84	87
Aerated soil at 40% WHC; KNO_3 and K_2HPO_4 in presence of Sphingomonas and Azospirillum		100	100	81	90
Aerated soil at 40% WHC; nutrients; biosurfactant MAT10		100	100	79	90
Aerated soil at 40% WHC; nutrients; ferric ion added as ferric octoate		100	100	87	88

2. Enzymes degrading polycyclic aromatic hydrocarbons

In an effort to remove as many PAHs as possible in the shortest time, many researchers are trying to discover new enzymes or strands in wild-type bacteria with the aim of engineering or immobilising them on opportune devices for bioremediation. This research has two aspects, the first being the search for new bacteria or enzymes able to degrade PAHs over a broad spectrum of chemical conditions. Table 1 reports the results obtained by Arun et al. [1] regarding various bacteria and conversion efficiencies; interestingly, as shown, when bacteria are mixed, the conversion rates decrease. The second aspect is the attempt to develop experimental conditions or molecular stand-ins as mediators to increase the degradation efficiency of target molecules. In particular, Arun's [1] work clearly notes the need for more targeted studies on enzyme bioremediation and any external factors that influence it. For example, it is not clear why mixing enzymes in some cases greatly reduces PAH removal efficiencies but in others has no effect. For example *Pseudomonas* sp. (see Table 1) can degrade 95% of available pyrene and *Pleurotus ostreatus* can degrade 32% of pyrene, yet by mixing the two bacteria, only 17% pyrene is degraded. The study by Arun et al. highlights how triggering chemical parameters may alter the degradation activity of bacteria; in particular, it is expected that the mixture of more than one type of bacterium affects the degradation of small molecules, ions or enzyme–enzyme interactions, as reported by Vinas et al. [12]. Vinas et al. studied a highly creosote-contaminated soil, observing that the addition of nutrients, moisture content and aeration were the key factors of PAH bioremediation, as reported in Table 1; however, it was evidenced that there was a remarkable difference in the composition of the bacterial community. Moreover, there are other parameters that could be necessary to consider, such interspecies interactions, nutrient effects, changes in PAH bioavailability and recalcitrant effects. Along with performing studies on bacteria, strain types and mixtures thereof, many authors have tried to study and characterise new enzymes.

2.1. Environmental and chemico-physical parameters affecting degradation

A pivotal aspect of enzymatic degradation that in many cases can explain abnormal efficiency, reaction kinetics or selectivity is represented by chemico-physical parameters and other variables affecting PAH degradation [13]. One of these variables is the aerobic condition of the reaction; in particular, it is well known that the removal of PAHs under anaerobic conditions is normally two-fold less efficient than that under aerobic conditions (Table 2). However, McNally et al. shed light on PAH bioremediation also being possible under severe conditions such as anaerobic conditions [14]. McNally reports three pseudomonad strains with activities, which are in some cases analogous to aerobic strains. The time required for the colonisation of a culture medium by the appropriate degradation organism is critical, but once this step is reached, PAH conversion is as quick as that under aerobic conditions. Though aerobic conditions appear to be pivotal for PAH removal, slight changes in structure may overcome external degrading conditions. This idea was developed by Shuler et al. [15]. Shule et al. discovered a new pool of strains from *Sphingomonas* sp. that can oxidise low-molecular-weight PAHs, chlorinated biphenyls, dibenzo-p-dioxin and high-molecular-weight PAHs such as benz(a)anthracene, chrysene and pyrene. The authors hypothesise that a pool of highly conserved multi-component dioxygenases that exhibit slight structural variations in their amino acid sequences outside the catalytic pocket may appear to be responsible for larges differences in selectivity towards PAHs. Another chemical parameter with a degradation-modulating effect was reported by Mancini et al. [16], who suggested that PAH degradation may be modulated by trace iron elements [17]. The authors conducted experiments on the modulation of iron ion concentrations ([Fe]/[Toluene] = 10-1 and 10-3), optimising the corresponding isotope analysis to obtain evidence of the significant effect of these trace elements; however, it is clear that further study is required (Table 2). One of Mancini's experiments shows that the trace element cobalt is required to drive the reductive dechlorination of chlorinated ethenes by vitamin B12; hence, examining the effects of cobalt limitations on enzymatic activity and isotopic fractionation is warranted. The possible mechanism governing the activity of iron ions towards PAH degradation was studied by Santos et al. [18]. The researchers established that iron ions enhance anthracene degradation directly by increasing the activity of the enzymes involved in the aerobic biodegradation (Table 2) pathways of hydrocarbons and indirectly by increasing PAH solubility due to stimulation through biosurfactant production [14,19]. Furthermore, Santos et al. noted that iron ion activity directly correlates with salt solubility, Fe_2O_3 being less active than $Fe(NO_3)_3$. Although the extent of degradation increases, it is not accompanied by a significant change in the degradation rate, indicating a new possible degradation pathway. It is noted that the proteins in the active site of the degrading enzyme usually contain several iron ions, such proteins being commonly categorised as non-heme iron proteins. These enzymes form a broad class of molecules that exhibit a Rieske centre. The presence of iron ions at the active site of this class of protein supports the hypothesis that external iron ions may be involved in enzymatic activity in an unknown way. If this hypothesis is confirmed by

Table 2
Chemical parameters influencing PAH degradation.

Enzyme or strain or molecule studied	Parameter	Effect	Reference
Pseudomonas	Aerobic condition	Anaerobic conditions is normally two-fold less efficient than in aerobic conditions	McNally et al. [14]
Pseudomonas putida	Iron ions	Kinetic of degradation	Mancini et al. [16]
Pseudomonas sp.	Iron ions	Conversion rate	Santos et al. [18]
Laccase	Presence of: vanillin, acetovanillone, acetosyringone, syringaldehyde, 2,4,6-trimethylphenol and p-coumaric acid	Conversion rate	Canas et al. [35]
Cytocrome P450	pH < 3	Conversion rate	Sood and Lal [20]
PAH only	Solubility	Conversion rate	Castelli et al. [24]
PAH only	Solubility	Conversion rate	Eibes et al. [25]
PAH only	Medium composition and solubility	Conversion rate	Vinas et al. [12]
Laccase	Presence of: 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid, diammonium salt (ABTS) and 1-hydroxybenzotriazole HBT)	Conversion rate	Farnet et al. [31]

other studies, the iron ion may be recognised as a crucial factor in bioremediation, on the one hand increasing the solubility of recalcitrant PAH and on the other opening the possibility of hitherto unexplored degradation pathways. Another parameter that is critical to the efficiency of many degradation reactions is pH level. In particular, Sood and Lal [20] have revealed novel yeast species isolated from soil samples contaminated with acidic oily sludge (pH 1–3) that can degrade 73% of total petroleum hydrocarbons at pH 3 within a week. In this case, an enzyme with 60% homology to cytochrome P-450, one of the first enzymes studied for bioremediation [21,22]. Again, environmental conditions (pH) determine enzyme activity. Additionally, the author discovered that this new strain could function under worse conditions typical of real applications; thus, more studies are required to better understand the defense mechanisms of this strain against pH. In many of the cases reported herein, the first step of degradation is the vehiculation of PAH to the enzyme or bacterium. This step represents, in most cases, the slowest step. A recent study in this direction was reported by Castelli et al. [23,24], who used calorimetry to study degradation. In particular, the researchers discovered that PAHs could interact with model membranes but were unable to migrate through an aqueous medium to reach biological membranes (Table 2). Furthermore, PAHs can be transferred from loaded vesicles to empty large unilamellar vesicles (LUVs). These results suggest that lipophilic agents favour absorption such that these interactions should correlate with other mechanisms caused by the transfer from a lipophilic medium to a biological membrane. The possibility of using LUVs as carriers should obviate this transfer, leading to a great increase in PAH degradation. Another attempt to overcame the solubilisation step of PAH degradation was reported by Eibes et al. [25]. The authors tried to enhance the solubility of PAHs by using an acetone solution (36%, v/v). The results clearly demonstrate great extents of degradation (greater than 95%) for anthracene, dibenzothiophene and pyrene achieved in less than 24 h (Table 2). Moreover, the authors shed light on these degradation mechanisms by analysing degradation residues. The study highlighted an undocumented mechanism for dibenzothiophene (Fig. 1 panel b) with an intermediate that breaks PAH into a simpler acid and pyrene (Fig. 1 panel a). In addition to the studies already discussed, an attempt to understand and increase the solubility of PAHs in an aqueous environment (Table 2) was reported in a recent paper by Vacha et al. [26]. The authors investigated the adsorption of benzene, naphthalene, anthracene and phenanthrene at the water interface using molecular dynamics simulations. In that paper, MD simulations were employed to reconstruct the potential mean force (PMF) profile for the process of transforming from a gas phase to an aqueous one. The results indicate that, in all cases, a deep minimum in the free energy profile corresponding to the water-gas interface occurs. This finding points to the importance of the aqueous surface for the chemistry of PAHs. The free energy minima of PAH molecules at the air–water interface imply that in cases in which the surface area is large, the surface reactivity of PAH molecules can be more significant than the bulk chemistry, such as in atmospheric droplets, ice, snow and thin water films on aerosols.

2.2. Manganese peroxidase

In a study on highly efficient degrading enzymes, Hofrichter et al. [27] investigated manganese peroxidase (MnP) from *Nematoloma frowardii* to shed light on the concept of enzymatic combustion [28], discovering a molecule that is able to degrade a broad range of pollutants such as 2,4,6-trinitrotoluene and catechol. Despite this degrading ability, MnP is unable to degrade some PAHs such as pyrene and in addition, its degradation mechanism is not well understood. Notwithstanding these difficulties, the availability of the well-resolved structure of MnP makes this enzyme attractive for applied research in this field. In fact, many studies focus on exploiting enzyme mutation [29] to increase the efficiency of MnP and better understand PAH-enzyme interactions. Zang et al. obtained some mutants of MnP by applying site-directed mutagenesis to arg42 and ASN131. The results revealed some species with different degrading activities, but the lack of accurate engineering strategies and characterisation of mutants has prevented researchers from obtaining information that is more useful. Although the work of Zang is one of the first efforts in this meaningful direction, other researchers have explored the route of immobilisation, which represents the first step in the search for versatile applications of this enzyme. Acevedo et al. [30] obtained immobilised enzymes with higher efficiency than free enzymes. After 24 h, manganese peroxidase (MnP) immobilised on nano-clay efficiently transformed anthracene and pyrene into anthraquinone and 4,5-dihydropyrene, respectively and, to a lesser extent, fluoranthene and phenanthrene. Immobilised MnP was generally twice as efficient as free MnP. As evidenced in this review, Acevedo drew attention to the environmental factors affecting degradation.

2.3. Laccase

Farnet et al. focused on six different isoforms of laccase from *Marasmius quercophilus* [31] that were able to oxidise widespread pollutants, such as PAHs. Though many crystallographic structures of laccase are available, Farnet et al. lacked this information in their study, but their work is reported in this study because it provides useful hints regarding degradation that we believe may be

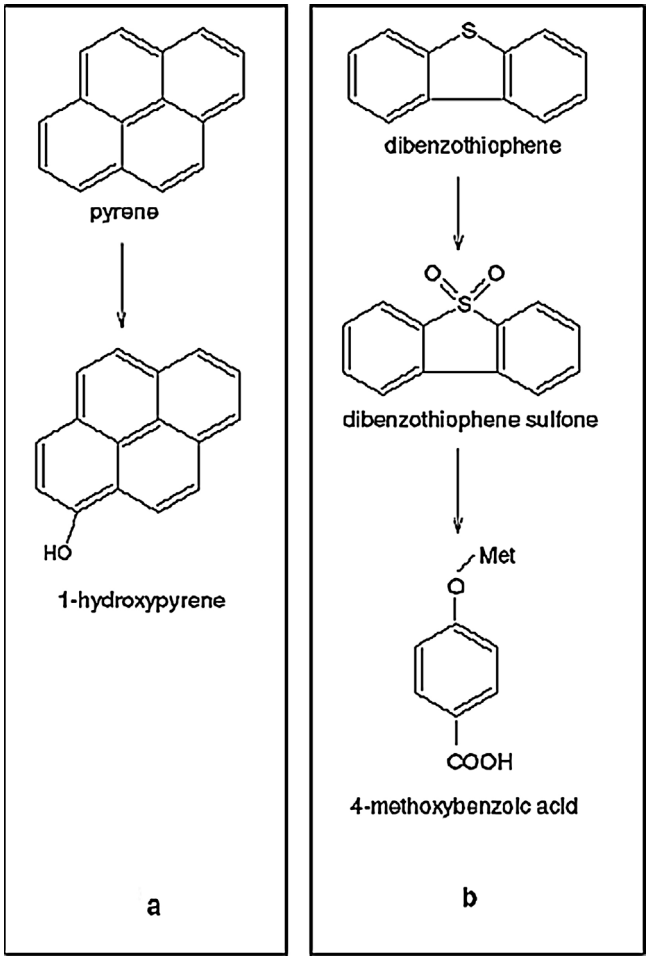

Fig. 1. Pyrene (a), dibenzothiophene (b) and their oxidation products [26].

applied to other degrading enzymes. In vitro studies confirmed that these laccase isoforms are able to transform anthracene and benzo(a)pyrene, though naphthalene and phenanthrene were not degraded. In the same work, Farnet et al. noted that the conversion of anthracene to anthraquinones may be greatly enhanced by using 2,2′-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS [32]) and 1-hydroxybenzotriazole (HBT), which act as electron transporters for laccase. Furthermore, this study revealed that the determining factor for oxidation was the ionisation potential (IP); indeed, only molecules with an IP < 7.55 eV were degraded. Unfortunately, though dozens of laccase structures are currently known, no 3D structures of this specific enzyme have been investigated. Although it is remarkable that enzymes of the same class may have exhibit different responses to degradation, an approach based on 3D structure may lead to the possibility of engineering enzymes that are specifically designed for bioremediation. As reported for MnP, immobilisation represents the first step in the commercialisation of enzymes and some authors are currently pursuing this step. However, although laccases are promising enzymes [33,34] for degrading PAHs, their efficiency decreases rapidly during the industrialised process of immobilisation. In a recent study, Hu [33] immobilised one isoform of laccase from *Trametes versicolor* on silica nanoparticles with the aim of developing an efficient industrial application for this system such that when it was immobilised it became less active due to partial unfolding or underwent general structural destabilisation. In many studies, it has been demonstrated that immobilised enzymes are able to degrade anthracene (ANT) to

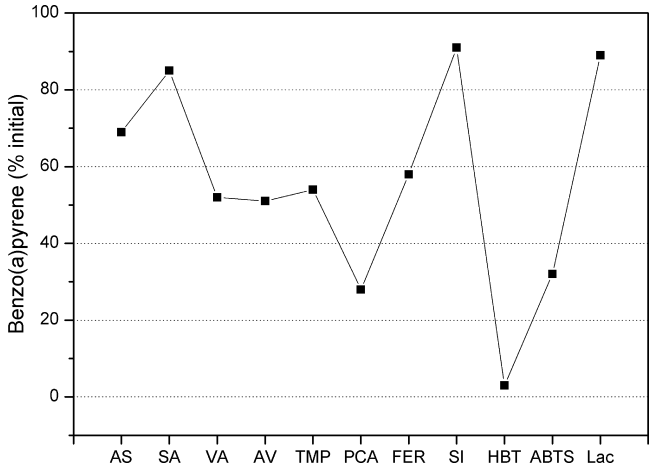

Fig. 2. Residual benzo[a]pyrene (in % of initial 50 M) after 24-h oxidation with laccase and different mediators (500 M). Mean values and 95% confidence limits are shown. Mediators: acetosyringone (AS), syringaldehyde (SA), vanillin (VA), acetovanillone (AV), 2,4,6-trimethylphenol (TMP), p-coumaric acid (PCA), ferulic acid (FER), sinapic acid (SI), HBT, and ABTS (Lac, laccase without mediator) [36].

anthraquinone (ANQ) via a pH-dependent mechanism. Considering the evident differences in efficiency between laccase and MnP, as reported above, a comparative study of the different enzyme–substrate interactions should prove interesting, with the objective of minimising the altered functions of the immobilised enzymes. Moreover, the concept of using a mediator in enzyme degradation may be relevant to industrial applications, especially for minimising costs. Indeed, mediators may become crucially important in future studies on the mechanisms involved in the use of mediator activities as tools for optimising enzyme activity. In a recent work by Canas et al., the authors provided evidence that chemical conditions are very important in determining PAH degradation, particularly for laccase [35]. Such enzymes have an in vitro conversion rate of approximately 15%, but with other molecules such as vanillin, acetovanillone, acetosyringone, syringaldehyde, 2,4,6-trimethylphenol and p-coumaric acid, their activity is greatly enhanced (Fig. 2). The mechanism of mediated oxidation is not yet clear, although different routes are believed to be involved depending on the mediator involved [36].

2.4. Soybean peroxidase

The same observations discussed above have been reported by other authors in studying an enzyme that has long been known to be involved in PAH degradation, soybean peroxidase (SBP). SBP catalyses the oxidation of a variety of PAHs but only if the pollutants are dissolved in organic solvents such as acetonitrile, tetrahydrofuran, or dimethyl formamide. Another shortcoming of SBP [37] is the limited pH range (2–2.5) over which it functions efficiently. Within this range, the conversion rate of SBP is very high (>90%) and despite its limitations, this enzyme is a good choice because of its efficiency. There have been many attempts to overcome the limitations of free SBP [38,39] but to no avail; thus, further study is required. Nevertheless, SBP immobilisation is a well-known and efficient degradation route.

2.5. Naphthalene dioxygenase

To date, the only PAH dioxygenase used in industrial applications is naphthalene dioxygenase (NDO) [40]. McIver reports that NDO is well suited to producing intermediates for the chemical industry; for example, diols produced from PAH degradation represent an ideal starting point for many chemical syntheses. Moreover,

NDO is very interesting because of its degradation of a broad range of pollutants and it was one of the first molecules discovered to initiate bioremediation naturally. In many cases, researchers [40–42] have adopted NDO not only as a degradation tool but also as a biomarker. NDO activity is considered a precursor to natural biodegradation. All of the enzymes herein listed – MNP, SBP, NDO, Laccase and AKR – are bioremediable, but much work is needed to investigate how they operate in a complex matrix that is both biological and chemical. To better steer further studies, we collected significant data in Table 3; all selected enzymes have a known 3D structure, which is key to deeply understanding all of their characteristics and fundamental for further engineering the enzymes.

3. Polycyclic aromatic hydrocarbons: computational aspects

3.1. Bioinformatics methods

Studies on laccase have been directed towards the determination of the kinetics of degradation. Cristovao et al. [43,44] used a mathematical approach to determine the kinetic constants that adequately describe the degradation kinetics of some reactive textile dyes. The results were confirmed by comparing the time courses obtained experimentally with those obtained from the model the authors developed. These results may be used to predict the time courses of substrate consumption and product formation under different substrate concentrations. Thus, establishing kinetic models for these reactions is a useful tool for simulating and designing enzymatic bioreactors. Interestingly, the kinetic data calculated by the models coincide well with the experimental data. Although this work is not directly related to enzyme bioremediation, the method used to calculate kinetic data may be extended to other systems. While the techniques employed in such studies appear to be valid as a methodological approach and the results thus obtained are in good agreement with experimental data, we believe that a more in-depth approach should be applied to enzymatic systems to enhance our knowledge of enzyme–PAH interactions. Other bioinformatics methods, particularly electrostatic methods and experiments, were applied by Brown et al. [45] to determine the redox potential of NDO. The large range of reduction potentials for Rieske ferredoxins (from −150 to +400 mV) was first suspected to arise from the different extents of solvent accessibility to the cluster, but studies performed to determine the structure of Rieske ferredoxin proteins and related studies have demonstrated that there are no large variations in solvent accessibility. Subsequent observations indicate that differences in the electrostatic environment and not structural differences between Rieske proteins are responsible for the wide range of reduction potentials observed. Brown et al. developed a model to predict the reduction potential of Rieske proteins given only their crystal structure. The method proposed accurately predicts the reduction potentials of 17 Rieske proteins for which both the structures and experimentally determined potentials are available. Additionally, the method employed has a bright future in facilitating in silico prediction of the effects of mutations on Rieske protein reduction potentials and estimating reduction potentials for newly determined Rieske ferredoxin structures.

Table 4
GOLD average fitness scores for known substrates, a few predicted targets and newly predicted targets for bioremediation [47].

S. no.	Name	GOLD average fitness score	
		Trametes versicolor	*Bacillus subtilis*
1	ABTS	50.58	48.14
2	Anthracene	40.37	30.22
3	Phenanthrene	42.05	31.62
4	Thiodicarb	59.01	41.61
5	Malathion	57.29	48
6	Captan	44.23	39.27
7	Atrazine	44.24	30.29
8	Indigo	44.6	40.34
9	Remazol red B	47	33.5
10	Vanilic acid	31.86	–
11	2,4-Dichlorophenol	30.22	30.66
12	m-Chlorophenol	30.25	–
13	2,4,6-Trichlorophenol	32.17	31.94
14	Sinapic acid	37.67	–
15	Syringadazine	33.32	30.3

3.2. Docking calculations

A docking approach for laccase was proposed by Suresh et al. The researchers analysed the binding of laccase to a broad range of molecules to develop a useful tool for finding putative pollutants for other biodegrading enzymes [46]. The authors observed a good match between the predicted bioremediation of laccase and the results of experiments on anthracene and phenanthrene degradation (Table 4). The authors reported that, in some docking simulations, the experimental data diverged because of the scoring function used and metal-related problems in docking, which are further complicated by the difficulty of reproducing the multiple coordination geometries of the copper complex [47,48]. NDO was also studied using a docking approach. Carredano et al. [49] docked low-molecular-weight PAHs (indole, naphthalene and biphenyl) with NDO and the results of their study suggest the presence of pockets reserved for the binding of the aromatic ring. The probable binding site of dioxygen is located between this pocket and the catalytic iron. A similar approach using the same enzyme was adopted by Librando and Forte, who employed molecular dynamics and docking techniques to explore new structures similar to wild-type NDO [50]. The authors created a library of opportune fragments in silico and carried out MD and docking simulation. The simulation results for these fragments, accounting principally for energy parameters, produced a short list of peptides with strong

Table 3
Data for manganese peroxidase (MnP), soybean peroxidase (SbP), naphthalene dioxygenase (NDO), laccase.

Enzyme	PDB id.	Degradation capability	Immobilisation	Reference
MnP	1MNP	2,4,6-Trinitrotoluene and catechol but not pyrene	Degrade anthracene and pyrene	Kirk and Farrell [28], Acevedo et al. [30]
SbP	1FHF	Degrade a broad range of pollutants including PAH	–	Kraus et al. [37]
NDO	1O7H, 1O7G, 1O7 N, 1O7P, 1O7 W	Degrade a broad range of pollutants	–	Di Gennaro et al. [42] Wammer et al. [54]
Laccase	3KW7, 3FU7, 3FU8, 3FU9, 3DIV, 2ZWN, 3F8X, 3CG8, 4A2D, 4A2E, 4A2H, 2Q9o, 2QT6, 2HRG, 2HRH, 2H5U, 2IH8, 2IH9, 1V10, 1GYC, 1GW0, 1KYA, 1HFU, 1A65, 1UVW	Anthracene and benzo(a)pyrene but not naphthalene and phenanthrene	Degrade anthracene	Farnet et al. [31] Hu et al. [33]

Table 5
This table summarises theoretical calculation and experiments comparison on enzyme.

Enzyme	Technique	PAH docked	Experiments comparison	Reference
Laccase	Docking	Yes	Yes	Suresh et al. [48]
Laccase	Kinetic of degradation	No	Yes	Cristovao et al. [44]
NDO	DFT/AB initio	No	No	Librando and Alparone [59]
NDO	MD/docking	Yes	No	Librando and Forte [50]
PhnI	MD/docking	Yes	Yes	Jakoncic et al. [55], Librando and Pappalardo [57], Librando and Pappalardo [58]
Estrogen receptor α (enzyme adopted for functional studies)	MD/Docking/QSAR	Yes	No	Li et al. [61]

binding activity, which may be a great boon for future laboratory work. Further field studies of the same group [51] will increase the accuracy of the binding parameter and help generate a larger library by adopting hydrophobicity, free binding energy and RMSD as indicators to better highlight binding zones and modifications that are able to help NDO bind PAH efficiently. Moreover, this field of research generally utilises a database approach, but in this study, a database had to be generated specifically for the target enzyme. Such studies offer interesting opportunities for molecular screening to highlight active strands against PAH but also evidence the need to manage large databases of molecules and fragments, as addressed in the final part of this review. The first step in the biodegradation of aromatic hydrocarbons often involves the dihydroxylation of two adjacent carbon atoms on the aromatic ring, catalysed by ring-hydroxylating dioxygenase (RHD). Several bacteria have been found to degrade PAHs, but only a few have been reported to attack four- and five-ring PAHs [52–54]. Jakoncic et al. [55] indicate that the broad substrate specificity of the dioxygenase from *Sphingomonas* sp. Strain CHY-1i (PhnI) [56] is primarily due to the large volume and particular shape of the enzyme's catalytic pocket. Molecular simulations of the PhnI pocket revealed the pocket to be at least 2 Å longer and wider at the entrance, a unique feature of dioxygenases with known structure that certainly allows five-ring benzo(a)pyrene to bind to catalytic Fe. Modelling various PAHs shows that Phe 350 in the central region of the pocket is essential for regio- and substrate-specificity, whereas Leu 223 and Ile 260 in the distal region provide the specificity of high-molecular-weight PAHs. Further studies involving replacements for the specific residues of substrate-binding pockets by site-directed mutagenesis should provide new insight into the role of these residues in the catalytic activity of the enzyme. Along the same direction of research, the authors of the present review adopted similar strategies and extended their potential in targeting some amino acids that are interesting for future mutagenesis and indicate a shape factor in engineering enzymes that are able to degrade PAH [57,58]

3.3. Quantum mechanical techniques

One of the widest and most complete works on enzyme degradation was reported in 2006 by Wammer et al. [54]. The authors systematically collected PAHs able to interact with NDO and the reaction products thereof. The most interesting part of this work was the application of in silico bioremediation via DFT studies and modelling, the results of which indicate that thermodynamically all PAHs can interact with the active sites of NDO. For both enzymes and PAHs, only steric hindrance determines which molecules can efficiently react with NDO. To better comprehend PAH-enzyme interactions, a direct application of the structure/reactivity relationship was adopted by Librando and Alparone [59]. A mixture of ab initio and functional density theory calculations for a series of dimethylnaphthalene (DMN) isomers was adopted to predict the PAH degradation efficiency. The results support the idea that electronic polarisability may be a useful tool for predicting the biodegradation trends of a series of compounds, besides playing a fundamental role in the biodegradation process of DMNs and providing a theoretical basis for Farnet's hypothesis discussing at the beginning of this paper. Strictly related to the structure reactivity/relationship for an enzyme, the method was combined with docking techniques to effectively characterise PAH–NDO interactions.

The mechanism of the interactions between PAH and enzymes is not yet fully understood and it is likely that it will not be easily accessed using experimental techniques. Recent studies employing a quantitative structure–activity relationship (QSAR) approach indicate that some experimental tools may offer great help in this respect. Li F. et al. [60,61] investigated the binding interactions between PAHs and various substrates such as DNA or oestrogen receptors. The descriptors incorporated into the QSAR models indicated that the binding activity was related to molecular size, van der Waals volume, shape profile, polarisability and electrotopological state, hydrogen bonding, hydrophobicity and p–p interactions. In those studies, QSAR was adopted to screen mutations, in silico, that improve enzyme reactivity. Moreover, QSAR has been applied to provide information about the toxicity of PAHs and any degradation intermediates [62], revealing that a significant relationship exists between toxicity and lipophilicity (K_{ow}), which suggests that non-polar narcosis is the prevalent toxic effect of the tested PAHs. This result is observed because toxicity, which is directly related to lipophilicity for biological membranes (i.e., non-polar narcosis), depends mostly on the amount of the compound accumulated in the same membranes. In addition, the ionisation potential of PAHs has been identified as an important parameter in explaining their toxic effects in terms of their $\log K_{ow}$.

3.4. Database approach

The enormous amount of data regarding reaction environment and degradation creates a serious problem in finding the right reaction, degradation products, etc. required. Thus, a database approach may be useful. One complication associated with the database approach is the integration of heterogeneous sources of information related to bioremediation. In this respect, the work by Pazos et al. offers a troubleshooting 'metarouter' to help in such cases [63], which is a useful instrument for assessing the environmental fate of compounds or mixtures and designing biodegradative strategies for these species. For chemical compounds, the following information is provided: name, synonyms, SMILES code, molecular weight, chemical formula, image of the chemical structure, canonical three-dimensional structure in PDB format, physicochemical properties (density, evaporation rate, melting point, boiling point and water solubility—the user can define and input new ones) and links to other databases. For reactions, the following information is provided: substrates and products, catalysing enzyme and links to other databases. For enzymes, the following information is provided: name, Enzyme Commission (EC) code, organisms where the gene is present, database sequence identifiers and links to other databases. The limitation of the metarouter is that it focuses on the

biochemical aspects of biodegradation rather than the nature of the biomolecules carrying out the reactions. In a recent study, Carbajosa presented a new database, Bionemo [64], which is a resource that complements other biodegradation databases. Bionemo was built by manually associating data from published articles and, in general, from the biodegradation literature and linking them to an underlying biochemical network. Currently, Bionemo contains sequence information for 324 reactions and transcription regulation information for more than 100 promoters and 100 transcription factors. Meanwhile, current biodegradation databases link reactions to protein sequences in databases that have been annotated with the corresponding EC codes. However, this method may be inaccurate. For instance, many reactions share the same EC code, although they use distinct substrates and generate different products. The Bionemo database combines metabolic, genetic and regulatory information. The central entries of the database are enzymatic complexes. These are linked to biochemical reactions that transform substrates into products. Reactions are associated with different pathways.

3.5. Conclusions: theoretical methods can support experiments

The objective of this review was to provide an overview of notably different problems, such as those associated with the organisation of bioremediation research, problems regarding enzyme activity, MD, docking and DFT; these problems, however, have several features in common. Bioremediation pathways are ultimately reduced to processes that remove PAHs and feature complex biological matrices and various chemical conditions such as pH, metal ions, etc. In this paper, we have presented select evidence regarding many new enzymes with known 3D structures obtained from X-ray or NMR, as well as highlighted important aspects regarding the removal of PAHs. Although experimental approaches provide highly reliable data that are not comparable to those yielded by many in silico methods, theoretical approaches offer certain advantages. For example, Carredano [49] produced mutants by site-directed mutagenesis to study the effect of individual amino acids on the degradation activities of NDO, undeniably demonstrating that the cost of such experiments is great.

Today, with the modern techniques of modelling and high-performance computing centres, such experiments could be considerably improved by performing prescreening studies, such as the study performed by the authors of this review in the case of the enzyme PhnI. Similarly to Carredano, Librando studied the effect of the mutation of single amino acids on the affinity of enzymes for some molecules of environmental interest. Of course, pairing MD with docking techniques will not replace but supplement studies on site-specific mutagenesis and experimental approaches in general by reducing the number of experiments that must be performed in vitro and consequently costs.

Other theoretical studies involving DFT and QSAR may boost current studies in the field of bioremediation by both shortening the time required to obtain new chimerical enzymes and reducing the number of laboratory experiments that must be performed. Other useful techniques that could supplement experiments include quantum mechanical (QM) techniques, which can predict the reactivity of receptor molecules and in some cases also some characteristics of substrates. Of course, these techniques can be applied only to small systems due to the high complexity of the calculations. In this context, QM methods can be adopted as methods supporting MD/docking, offering a vision of effects that would otherwise not be accessible. Finally, the use of databases makes it possible to accelerate the selection of molecules or reaction pathways through the use of intelligent algorithms. All theoretical studies regarding, for instance, kinetics, DFT, docking and molecular dynamics shed light on the great potential of in silico bioremediation. Moreover, the results of computer simulations used to study laccase agree well with corresponding experimental data, indicating a high degree of reliability (Table 5). The studies on laccase do not describe any enzyme mutations but offer interesting hints. NDO and PhnI are both good candidates for further studies and have been well characterised via in silico approaches. This work should offer a useful perspective of powerful in silico tools for incorporating structural modifications into selected enzymes.

Though it is not clear if there is a crucial factor for degradation, essential characteristics clearly include 3D structure, the presence of small molecules and chemical conditions. The present study will thus serve as an important reference in planning future experiments.

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