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The versatility of *Penicillium* species to degrade organic pollutants and its use for wastewater treatment

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Abstract

The removal of xenobiotics from industrial wastewater is of great interest to avoid environmental contamination. *Penicillium* species have been shown to be able to adapt its metabolism to many different circumstances and these fungi can use different xenobiotics as a carbon source. In this review, the ability of *Penicillium* to degrade different xenobiotic compounds is discussed. This review describes not only the biodegradation processes but also addresses the toxicity of the degradation products as well as the potential application of these processes in wastewater treatment. *Penicillium* strains have proven to be versatile and capable of being used for the biodegradation of different organic pollutants (phenols, azo dyes, hydrocarbons, pharmaceutical compounds, etc.) and show high potential to be used for wastewater treatment. From this review, it is concluded that beyond the degradation and optimization processes; pilot scale studies and toxicity must be carried out.

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Introduction

Wastewater generated by chemical, petrochemical, textile, resins, paper, leather, and glue, pharmaceutical and steel industries frequently contain high concentrations of xenobiotic compounds that represent a serious ecological problem due to their toxicity and widespread occurrence in the environment.

A xenobiotic compound is a chemical substance whose structure is rare or non-existent in nature as they are synthesized by humans in the laboratory. Xenobiotic compounds are also defined as substances that are present in concentrations much higher than usual and that would not be expected to be present within organisms. The discharge of wastewater containing different kinds of xenobiotics, into receiving water bodies endanger aquatic life, even at relatively low contaminant concentrations. Therefore, the removal of these xenobiotics from industrial wastewater is of great practical significance for environmental protection.

Several physicochemical and biological methods have been adapted for the treatment of different kinds of xenobiotics. In recent years, biological processes for xenobiotic degradation and wastewater reutilization have been developed including aerobic and anaerobic bacteria as well as fungi. There are many reports about the potential of filamentous fungi for sludge treatment which have been well described and reviewed by More et al.[1]. *Aspergillus niger* showed biodegradation and bioflocculation activities, arsenic bioremediation and bioconversion of olive mill waste. *Phanerochaete chrysosporium* showed biodegradation and bioflocculation activities, bioremediation of lignin, PCB's, PCP's and azo dyes. *Penicillium* (in particular *P. chrysogenum*) and *Paecilomyces* species showed pathogen removal, bioflocculation and biodegradation activities, and the removal of arsenic compounds and insecticides, among others.

In general, filamentous fungi have shown to be more tolerant to high concentrations of pollutants and they are less sensitive than bacteria and yeast to changes in their environment[1,2]. Some fungi tolerate extreme environmental conditions (temperatures of −5 to +60 °C; pH of 1 to 9) and grow at a water activity of only 0.65, or with 0.2% oxygen[3]. They are able to grow on low nitrogen medium, at low pH and low temperature[1]. In addition, they are easy to grow in fermenters and be separated by mechanical methods, due to their filamentous structure[2,4]. All these characteristics make them a promising alternative among various wastewater treatment technologies.

Among fungi, the most widely studied are the ligninolytic fungi or white rot basidiomycota fungi. However, these types of fungi often have two major limiting factors that hinder their applicability in industry: 1) they have high nutritional requirements (lignocellulosic substrate), and 2) many species have slow growth kinetics[5]. This encourages the study of other types of fungi[4–6]. Many non-basidiomycota fungi are also able to degrade aromatic compounds and other complex structures[2,6–12]. A good example of this kind of non-basidiomycota fungi is *Penicillium* sp., which belongs to the phylum Ascomycota.

Penicillium species are able to adapt their metabolism to many different environments, and are considered ubiquitous in nature, commonly found in food, indoor air and soil. In addition, they are among the most common fungi that spoil food and contaminate indoor environments[2,13,14]. Diversity and adaptable metabolism of *Penicillium* species allows them to survive in some of the most extreme environments on earth

including deep-sea sediments[15], polar regions[16,17] to the Himalayas[18], regions of extreme acidic pH[19] and in extreme temperatures[20]. Although, primarily categorized as decomposers, *Penicillium* are good hydrocarbon assimilators with low co-substrate requirements, and they can synthesize a of wide variety of biomolecules[2,13,14]. The use of various carbon sources demonstrates their capability to adapt to changing nutritional environments and their potential to decompose diverse materials.

There are many reports showing the ability of *Penicillium* to degrade various materials, including food waste, cellulose- and lignin-containing residues, and hydrocarbons[9,11,21,22] and to transform xenobiotic compounds into less mutagenic substances[6–8,23,24]. The occurrence of *Penicillium* spp. in sewage sludge has been reported[25]. In addition, *Penicillium corylophilum* was more efficient compared to *Aspergillus niger* for biodegradation of the domestic activated sludge, enhancing the sludge degradation rate by decreasing chemical oxygen demand (COD).

Filamentous fungi can grow on different matrices. In submerged culture, fungi can either grow in dispersed form or as spherical pellets consisting of aggregated hypha structures. Pellet morphology, process control and productivity are highly interlinked. The control process in a bioreactor usually requires compact and small pellets due to rheological issues[26]. For example, within *P. chrysogenum* pellets, problems with internal transport of substrates and products may occur, depending on size and compactness of pellets[27]. Cronenberg et al.[28], reported the formation of pellets with a diameter of less than 400 μm by *P. chrysogenum,* where the mass transfer resistance will be very low in these pellets, being an advantage for the wastewater treatment process. Moreover, the immobilization of *P. chrysogenum* on loofah showed a significant increase of azo dye degradation rate, with respect to the free cells[4]. Both, the immobilization and the pellet formation, leads to the possibility of biomass reuse and simplifies the operation for downstream processes.

All the features mentioned above make *Penicillium* particularly suitable to be used in wastewater treatment and degradation of organic pollutants. In this review, a summary of the capabilities of some species of *Penicillium* to degrade different toxic compounds are described and the analysis of its potential use for wastewater treatment is discussed.

Phenol and chlorophenols

Phenol and its derivatives are widely distributed as environmental pollutants due to their presence in the effluents of many industrial processes like chemical, petrochemical, steel, pulp and paper mill industries[2]. These effluents frequently contain high concentrations of phenolics compounds that represents a serious ecological problem due to their widespread use, toxicity and occurrence throughout the environment. Many phenolic compounds are hazardous, toxic, endocrine disrupting, mutagenic, teratogenic, and/or carcinogenic[29]. Therefore, the removal of phenol and its derivatives from industrial wastewater is of great practical significance for environmental protection. Moreover, chlorophenols have been introduced into the environment through their use as biocides, for example penta chlorophenol (PCP), trichlorophenol (TCP) and tectrachloro phenol (TeCP) were used historically as fungicides in wood-preservative formulations[30,31].

The biodegradation of phenols and chlorophenols by *Penicillium* species has been reported since the 90's and several works have continued studys in this regard (Table 1). In 1993, Hofrichter et al.[32] reported a *Penicillium* strain (Bi 7/2) able to grow on phenol (1,000 mg·l^{-1}) as sole source of carbon and energy, and metabolized the phenol by the ortho-pathway. This strain also metabolizes 4-, 3- and 2-chlorophenol (50 mg·l^{-1}) and 4-, 3- and 2-nitrophenol (50 mg·l^{-1}), with phenol or glucose as co-substrate. The fact that an external carbon source, such as glucose, is needed implies an additional cost for the process. However, many *Penicillium* species can use phenol as a carbon source. This facilitates the development of a treatment process, since most of the effluents that contain chlorophenols, for example effluents from pulp and paper mill industries, contains phenol that can be utilized as a carbon source. Later, Marr et al.[33] found a *Penicllium simplicissimum* SK9117 strain able to degrade 3-chlorophenol, 4-chlorophenol, 4-bromophenol, 3-fluorophenol and 4-fluorophenol. However, monobromophenols and monochlorophenols were transformed to other intermediates (chlorohydroquinone, 4-chlorocatechol, 4-chloro-1,2,3-trihydroxybenzene, and 5-chloro-1,2,3-trihydroxybenzene) and could not support the fungus growth as the sole carbon and energy source, while monofluorophenols were mineralized completely without a co-substrate. In addition, difluorophenols were transformed by *P. frequentans* strain Bi 7/2, using phenol as a sole source of carbon and energy[35]. From the 90's onwards, even up to 2021, more species of *Penicillium* were described with the ability to degrade phenol and chlorophenols (Table 1).

Table 1. Degradation of phenol and its derivatives by *Penicillium* spp.

Chemical compound	External carbon source	*Penicillium* spp.	Reference
Phenol	None	*P. frequentans* Bi 7/2	[32]
		P. chrysogenum var. *halophenolicum*	[23]
		P. chrysogenum ERK1	[8, 37]
		P. notatum	[41]
Resorcinol	None	*P. chrysogenum* var. *halophenolicum*	[24, 36]
Catechol, Hydroquinone	None	*P. chrysogenum* var. *halophenolicum*	[36]
2-chlorophenol	Phenol	*P. frequentans* Bi 7/2	[32]
	Acetate	*P. camemberti*	[39]
3-chlorophenol	Phenol	*P. frequentans* Bi 7/2	[32]
		P. simplicissimum	[33]
4-chlorophenol	Phenol	*P. frequentans* Bi 7/2	[32]
		P. simplicissimum	[33]
2-nitrophenol	Phenol	*P. frequentans* Bi 7/2	[32]
3-nitrophenol	Phenol	*P. frequentans* Bi 7/2	[32]
4-nitrophenol	Phenol	*P. frequentans* Bi 7/2	[32]
4-bromophenol	Phenol	*P. simplicissimum*	[33]
3-fluorophenol	None	*P. simplicissimum*	[33]
4-fluorophenol	None	*P. simplicissimum*	[33]
2,3- difluorophenol	Phenol	*P. frequentans* Bi 7/2	[35]
2,4- difluorophenol	Phenol	*P. frequentans* Bi 7/2	[35]
2,5- difluorophenol	Phenol	*P. frequentans* Bi 7/2	[35]
3,4- difluorophenol	Phenol	*P. frequentans* Bi 7/2	[35]
2,4,6-trichlorophenol	Acetate	*P. chrysogenum* ERK1	[7]
Pentachlorophenol	Acetate	*P. camemberti*	[39]
3,5-dimethyl-2,4-dichlorophenol	None	*Penicillium* spp	[40]

A case worth mentioning is that described by Leitão et al.[23], where a *Penicillium chrysogenum* var. *halophenolicum* was able to mineralize phenol completely at 5.8% NaCl, since this fungus was found to be halotolerant. This condition increases the chances to use this strain in biological treatments of phenol-containing wastewater, since some of them contain high concentrations of salts. The same strain degraded up to 250 $mg{\cdot}l^{-1}$ of resorcinol, as the sole carbon source in batch experiments in the presence of 58.5 $g{\cdot}l^{-1}$ of sodium chloride[24]. In addition, the authors showed the decrease of the acute toxicity of phenol and resorcinol, on *Artemia franciscana* larvae, after the bioremediation process with *P. chrysogenum* var. *halophenolicum*. Ferreira-Guedes & Leitão[36], described the removal efficiency of hydroquinone, catechol and resorcinol in binary substrate systems under saline conditions by the same *P. chrysogenum* var. *halophenolicum* strain. Catechol, resorcinol and hydroquinone are dihydroxybenzene isomers. The simultaneous presence of two or three isomers in a mixture will be defined as binary or ternary mixtures. The results of Ferreira-Guedes & Leitão[36] showed that the efficiency to remove dihydroxybenzene in binary substrate systems was higher than in mono substrate systems, except for hydroquinone. In the binary substrate systems, dihydroxybenzenes were removed not only simultaneously, but also preferentially. At high dihydroxybenzene concentration, fungal strain preferentially degraded hydroquinone followed by catechol and resorcinol.

Most of the results reported in Table 1, were obtained in batch culture in shaking conditions between 80 to 160 rpm. However, some studies showed that *Penicillium frequentans* Bi 7/2 and *Penicillium chrysogeunm* ERK1 could degrade dichlorophenols and phenol, respectively in resting mycelium conditions[35,37]. This may be convenient in terms of reducing the costs of wastewater treatment processes.

Furthermore, Aranciaga et al.[7] studied the biodegradation of 2,4,6-trichlorophenol, demonstrating that *Penicillium chrysogenum* ERK1 was able to degrade 85% of TCP in batch cultures in the presence of sodium acetate. In their study, hydroquinone and benzo quinone were identified as degradation intermediates, and although the complete mineralization of the TCP did not occur, a reduction on the phytotoxicity (50% approximately) was observed. The extent of degradation depends on the structure of the compound, the number of chlorine substituents, and the position of chlorine in the compound[38]. This directly influences the toxicity of the compound, which generally increases as the chlorinated substituents number increases. That is why it is equally important to reduce the toxicity of the effluent, even when the compound cannot be completely mineralized.

In the case of pentachlorophenol (PCP), Taseli & Gokcay[39] showed that *Penicillium camemberti* was able to remove 56% of PCP in batch experiments with acetate as a carbon source. In other experiments, without acetate but in the presence of Tween 80, *P. camemberti* removed 86% of the PCP. Moreover, an up-flow column reactor was operated with this fungus in the laboratory[39] and 77% of PCP removal was achieved in 4 d of contact without aeration and with minimum amount of carbon supplement. The percentages of PCP removal continued decreasing to 18.8% until the 18th day. These results agree with the results mentioned above, and show almost ideal conditions with respect to operating costs, without aeration and a reduced concentration of external carbon source.

In another study, Yan et al.[40] studied the performance of a *Penicillium* sp. strain to remove a 3,5-dimethyl-2,4-dichlorophenol (DCMX) from saline industrial wastewater. The results of batch experiments showed that biodegradation of DCMX was affected by pH value, salinity and DCMX concentration. The maximum DCMX removal efficiency was obtained at salinity 2.6%, temperature 32 °C and pH 5.87.

Dyes and pigments

The term colorant, which includes dyes and pigments, refers to substances capable of colouring a substrate. Colorants are used in industries like clothing, paints, plastics, photographs, prints and ceramics. They are used alone or in combination with other ingredients, which impart or alter the colour of the product[42]. Most dyes used in these processes are synthetic and are classified based on chromophore structures (namely acidic, basic, disperse, reactive, azo dyes and anthraquinone).

Dye wastewater treatment, mainly from textile industries, is really important in order to control its negative impact on the environment. Some dye precursors or its degradation byproducts were reported as toxic, carcinogenic and mutagenic[43,44], like aromatic amines which damage the DNA in cells and this leads to a risk of cancer[42].

The mycoremediation of dyes has shown to be a possible option to the conventional physico-chemical treatment technologies. The most widely used fungi in decolorization and degradation of dyes are the lignolytic fungi of class Basidiomycetes. However, non basidiomycotas fungi such as *Aspergillus niger* and *A. terreus*[45], *Rhizopus oryzae*[46] and some species of *Penicillium*[39,47–49] can also decolorize and/or biosorb diverse dyes[50,51].

For example, Shedbalkar et al.[47] showed that *Penicillium ochrochloron* decolorized cotton blue (50 $mg{\cdot}l^{-1}$), a triphenylmethane dye (Table 2). In this case, the dye was degraded to sulphonamide and triphenylmethane, as final products, by a battery of enzymes (lignin peroxidase, tyrosinase and aminopyrine N-demethylase) and the analysis of the phytotoxicity and microbial toxicity of extracted metabolites, suggested a decrease in their toxicity. The same *P. ochrochloron* has been shown to detoxify malachite green into p-benzyl-N,N-dimethylaniline and N,N-dimethyl-aniline hydrochloride. These metabolites were nontoxic when tested on *Triticum aestivum* and *Ervum lens* Linn (Table 2)[48]. The reaction was mediated by lignin peroxidase and the fungal culture was also found to have detoxified the textile effluent, reducing the values of total dissolved solids (TDS), total suspended solids (TSS), biochemical oxygen demand (BOD), and chemical oxygen demand (COD). In both works, it was demonstrated that *P. ochrochloron* was able to degrade and reduce the toxicity of two different dyes. However, it would be interesting to study the degradation and the analysis of the toxicity of the mixture of both dyes.

Moreover, *Penicillium simplicissimum* INCQS 40211 decolorized the textile dyes: Reactive Red 198 (RR198), Reactive Blue 214 (RB214), Reactive Blue 21 (RB21) and their mixture[52]. In this case, it was suggested that dye decolorization involved dye adsorption by the biomass first, followed by degradation. In addition, *P. simplicissimum* reduced the toxicity of RB21 from moderately acutely toxic to minor acutely toxic and it also reduced the toxicity of RB214 and the mixture of the three dyes, which remained minor acutely toxic. It is also worth

Table 2. Dye decolorization and degradation by *Penicillium* spp.

Penicillium spp	Chemical group	Dye name	Concentration ($mg \cdot l^{-1}$)	Toxicity analysis	Wastewater tested	Reference
P. chrysogenum	Azo	Direct Black 22, Direct Yellow 86, Direct Blue 200	200	*T. aestivum*	Diluted effluent	[4, 6]
P. ochrochloron	Triphenylmethane	Cotton blue	50	*T. aestivum* *E. lens* *A. vinelandii*	No	[47]
		Malachite green	50	*T. aestivum* *E. lens*	Diluted effluent	[48]
P. simplicissimum	Azo	Reactive Red 198 Reactive Blue 214	200	*D. pulex*	No	[52]
	Phthalocyanine	Reactive Blue 21	200	*D. pulex*	No	[52]
	Triphenylmethane	Methyl Violet, Crystal Violet, Malachite Green Cotton Blue	50–100	*V. radiate* *B. cereus* *S. aureus*	No	[53, 54]
P. oxalicum	Azo	Acid Red 183, Direct Blue 15 Direct Red 75	100–300	No	No	[5]
P. pinophilum	Triphenylmethane	Malachite Green	10	No	No	[55]

noticing that the fungus increased the toxicity of RR198. These results showed that more studies regarding dye degradation and toxicity reduction by *P. simplicissimum* INCQS 40211 are necessary. Later, Chen & Ting[53] and Chen et al.[54] described the biosorption and biodegradation activities of the same *Penicillium* species towards triphenylmethane dyes. Crystal Violet (CV), Methyl Violet (MV), Malachite Green (MG), and Cotton Blue (CB) were decolorized by *P. simplicissimum* with 98.7%, 97.5%, 97.1%, and 96.1 % of decolorization efficiency, respectively, within 2 h of incubation (50 $mg \cdot l^{-1}$, pH 5.0, 25 ± 2 °C) (Table 2). In this work, only UV–visible spectral analysis of dyes was conducted before and after treatment with *P. simplicissimum*, indicating the occurrence of biodegradation, however the intermediate products of the degradation or complete mineralization could not be confirmed. Some enzymatic activities were detected as manganese peroxidase, tyrosinase, triphenylmethane reductase activities, suggesting their involvement in the degradation pathway. In addition, reduction of phytotoxicity and microbial toxicity were observed only for MG.

Other *Penicillium* species that have been reported to have decolorization/degradation abilities are: *Penicillium oxalicum*[5], *Penicillium pinophilum*[55], *Penicillium purpurogenum*[56] and a *Penicillium* strain not characterized at the species level[57].

In all the cases mentioned above, degradation occurs with the addition of some external carbon source. In general, dyes are evaluated in culture media and only in a few cases mixtures of dyes and real effluents are studied.

Another strain, which is worth mentioning, is *Penicillium chrysogenum*. This fungus showed great potential to decolorize and degrade three azo dyes (at 200 $mg \cdot l^{-1}$) independently or a mixture of them, even in a complex wastewater matrix as it was real textile wastewater[6] (Table 2). The degradation process was carried out in the presence of glucose as a carbon source and showed that decolorization rates differed depending on the azo dye structure (number of azo bonds, terminal or substituent groups, steric hindrance, etc.). Moreover, a kinetic model for degradation was developed, which allowed prediction of the degradation kinetics of the mixture of the three azo dyes and the real textile wastewater[6]. Later, the immobilization on loofah of the same strain of *P. chrysogenum* significantly increased the degradation rate of DB22 in a laboratory scale as well as at bench scale reactor, with respect to the non-immobilized treatment[4]. The degradation rate of immobilized cells increased twice as compared to free-cells control and at day 5 the decolorization was almost complete, while without loofah, the total decolorization took more than 10 d. The results of these studies show an improvement in the azo dye degradation process, however, using glucose as a carbon source is still costly. Therefore, more studies should be carried out using alternative carbon sources such as waste from food industries, for example starch, beer bagasse, etc. to minimize effluent treatment costs.

Erdal & Taskin[58], also showed the potential of a strain of *P. chrysogenum* MT-6 to decolorize the textile dye Reactive Black-5. However, degradation was not confirmed in this case.

Lately, Fouda et al.[59] biosynthesized maghemite nanoparticles (γ-Fe_2O_3-NPs) using *Penicillium expansum* with the purpose of treating wastewater. Decolorization and degradation analyses, indicated that γ-Fe_2O_3-NPs was an effective biocatalyst for dye degradation under dose- and time-dependent manner. The highest decolorization (89%–90%) occurred after 6.0–8.0 h of incubation. The contaminant load of the textile wastewater was improved, as indicated by the reduction in COD, TDS, and TSS. Although, GC-MS results showed the complete disappearance of peaks in treated textile wastewater in comparison with the untreated samples, no toxicity analysis was carried out.

Pharmaceutical compounds

In recent years, the increase in the use and production of pharmaceutical compounds represent a potential environmental risk, since it could lead to antibiotic resistance, toxicity and can also cause endocrine disruption[10,12,60,61]. For this reason, a proper disposal and treatment or degradation of these compounds is necessary.

In this area, additional examples of biodegradation with *Penicillium* isolates can be found[10,12]. For example, the non-steroidal anti-inflammatory drug [2-(2,6-dichloroanilino) phenyl] acetic acid (Diclofenac; DFC) is used for the treatment of pain and inflammation, and it is one of the most widely used drugs around the world. It is considered as an emerging contaminant, being the number one persistent pharmaceutical substance in water bodies in 50 countries of the EU, Africa and America[62]. Olicón-Hernández et al.[10], were the first to describe the use of a *Penicillium* isolate able to transform DFC.

They studied DFC degradation by *Penicillium oxalicum* in flask and bench scale bioreactors, both with free and immobilized biomass. Pellets of *P. oxalicum* degraded 100 μM of DFC within 24 h, and the activity of CYP450 enzymes was the key for the drug elimination. The use of *P. oxalicum* reduced the acute toxicity of the medium supplemented with DFC, and the free biomass system exhibited the highest rate of DFC degradation in comparison with immobilized cells in the batch bioreactor. In addition, the same *Penicillium* isolate was able to reduce the concentration of other pharmaceutical active compounds, such as ketoprofen, naproxen and paracetamol in batch bench scale bioreactor in 24 h[61]. In general, the industrial effluents are not sterile and they usually have microorganisms, which can inhibit the growth and/or the degradation of toxic compounds by the degrading microorganisms that are of interest for wastewater treatment. For this reasons, the results obtained by Olicón-Hernández et al.[61] are of great importance since they showed that *P. oxalicum* inhibited the native fungal populations, present in the non-sterile real hospital wastewater, along with opportunistic human pathogens.

As it can be seen, in the case of DFC degradation, the immobilized cells did not improve the process, contrary to what was observed for the degradation of azo dyes with *P. chrysogenum*. For this reason, the treatment process of each effluent must be analysed independently to achieve optimal operating conditions.

Additionally, Li et al.[12], recently reported a *Penicillium oxalicum* strain that could efficiently degrade lincomycin (88.2% by day 6) from the antibiotic wastewater treatment plant and the fungal mycelium could be reused for at least ten batches with similar biodegradation efficiency. Besides, an endophytic strain of the same species could effectively degrade triclosan, which is an antibacterial and antifungal agent, into low toxic products[63].

These studies showed that *P. oxalicum* was able to reduce the concentration of pharmaceutical compounds in batch bench scale bioreactor, also it was not inhibited by the native fungal populations present in the effluent and the mycelium could be reused with good biodegradation efficiency. These characteristics strongly suggest that *P. oxalicum* has a high potential for the treatment of pharmaceutical compounds.

Hydrocarbons

Polycyclic aromatic hydrocarbons (PAHs) are poorly soluble, hydrophobic organic compounds which are among the most widely distributed organic contaminants[2]. They are released/transposed due to incomplete combustion of organic matter in petrochemical industries and proven to be highly genotoxic, mutagenic, carcinogenic as well as teratogenic to humans[64]. The PAHs are considered important environmental pollutants since they are the most frequently found in soil pollutants[65].

As described by Leitão[2] and Rabha & Jha[14], there are several reports regarding biodegradation of PAHs by *Penicillium* species[34,66–72] (Table 3). The effect of oxygen, ciclodextrins, surfactants, carbon and nitrogen sources, and other factors on PAHs biodegradation were studied in these reports. In addition, the presence of pyrene for example was described to influence the size and shape of the fungal pellets as well as the density of mycelium and hyphal length[71]. These studies, showed the degradation of different PAHs separately.

In 2014, Vanishree et al.[73] isolated a *Penicillium* sp. strain from petrol bunks soils and automobile workshops which can tolerate, grow and degrade different petrol concentrations.

Penicillium oxalicum was also reported to be able to completely remove anthracene and dibenzothiophene within 4 d, as well as phenanthrene and dibenzofuran, although at slower rates[75]. Most *Penicillium* strains which degrade PAHs carried out the degradation through the cytochrome P450 monooxygenase enzyme pathway. However, cytochrome P450 monooxygenase plays a role in the first steps of transformation of PAHs, while induction of oxygenase activity was detected in the subcellular fraction of the fungal mycelium exposed to these aromatic compounds.

Aranda et al.[75] demonstrated that glucose was required for anthracene degradation by *P. oxalicum* using a defined growth medium with low carbon content for stable isotope tracer experiments with ^{13}C 6-anthracene. Therefore, anthracene mineralization could not be confirmed, but ^{13}C-labelled oxy and hydroxy-derivatives were identified by nuclear magnetic resonance (NMR) as major metabolites. Although *P. oxalicum* was found to be the fungus with the highest and fastest PAHs degradation capability, the toxicity of these major metabolites should be evaluated, for a safe application in biotechnological pollutant removal processes.

Antarctic soil has also been a source of hydrocarbon degrading microorganisms[78,79] including *Penicillium*. A

Table 3. Hydrocarbon degradation by *Penicillium* spp.

Chemical compound	*Penicillium* spp	Reference
Acenaphthene	*Penicillium* sp. CHY-2	[74]
Anthracene	*P. oxalicum*	[75]
	P. ilerdanum	[76]
	P. oxalicum SYJ-1	[77]
Benzo[*a*]pyrene	*Penicillium* sp. CHY-2	[74]
	P. janthinellum	[66, 67]
Benz[*a*]antracene	*P. janthinellum*	[67]
Butylbenzene	*Penicillium* sp. CHY-2	[74]
Chrysene	*P. janthinellum*	[67]
Ethylbenzene	*Penicillium* sp. CHY-2	[74]
Dibenz[*a*,*h*]anthracene	*P. janthinellum*	[67]
Dibenzothiophene	*P. oxalicum*	[75]
Dibenzofuran	*P. oxalicum*	[75]
Fluorene	*P. italicum*	[69]
	P chrysogenum	[68]
Fluoranthene	*P. ilerdanum*	[76]
Naphthalene	*P. ilerdanum*	[76]
	Penicillium sp. CHY-2	[74]
Phenanthrene	*P. frequentans*	[72]
	P. ilerdanum	[76]
	P. oxalicum	[75]
	P. oxalicum SYJ-1	[77]
Pyrene	*P. simplicissimum*, *P. funiculosum*, *P. harzianum*, *P. terrestre*	[70]
	P. janthinellum,	[66,67,70]
	P. ochrochloron	[71]
	P. glabrum	[34]
	P. ilerdanum	[76]
	Penicillium oxalicum SYJ-1	[77]
Petrol	*Penicillium sp*	[73]
Decane	*Penicillium* sp. CHY-2	[74]
Dodecane	*Penicillium* sp. CHY-2	[74]
Octane	*Penicillium* sp. CHY-2	[74]

Penicillium sp. CHY-2 isolated from Antarctic soil was able to degrade not only aromatic hydrocarbons but also aliphatic hydrocarbons[74]. The highest level of degradation was for decane (49.0%), followed by butylbenzene (42.0%) and dodecane (33.0%), and lower levels of degradation were found for naphthalene (15.0%), acenaphthene (10.0%), octane (8.0%), ethylbenzene (4.0%), and benzo[a]pyrene (2.0%) at 20 °C. Later, the authors studied decane degradation in depth and showed that the addition of carbon sources such as glucose (5 g·l^{-1}) and Tween-80 (5 g·l^{-1}) enhanced decane degradation by about 1.8-fold and 1.61-fold respectively at 20 °C. 1,6-hexanediol was identified as one of the metabolites produced during the degradation of decane and a manganese peroxidase (MnP) enzyme was isolated from the fungi.

Over the years, more and more studies with new isolates able to degrade hydrocarbons have appeared, for example in 2021 a *Penicillium ilerdanum* NPDF1239-K3-F21, isolated from Arabian sea sediments, showed > 75% ability to degrade naphthalene, phenanthrene, pyrene, fluoranthene and anthracene[76]. However, beyond the degradation processes, further optimization, pilot scale and toxicity studies must be carried out before applying these processes to wastewater or bioremediation treatments.

Recently, Zhou et al.[77] showed a novel self-assembled PAH-degrading fungal mycelium *Penicillium oxalicum* SYJ-1-carbon nanotube (CNT) composites for pyrene removal. Their study is a good example of the combination of biodegradation and nanotechnology to increase the total PAH removal efficiency. Anthracene, phenanthrene and pyrene could be removed by 65%–92% within 72 h, while no naphthalene removal was observed by *Penicillium oxalicum* SYJ-1. Due to pyrene moderate degradation, this was selected as a model substrate to evaluate the possible positive effect of CNTs. The addition of it did not affect the growth of strain SYJ-1 and the complete removal of pyrene (20 mg·l^{-1}) was achieved within 48 h, while the sole fungus and CNTs alone could only remove 72% and 80% of pyrene at 72 h, respectively. Besides, the authors carried out a transcriptomic analysis, and a cytochrome P450 inhibition experiment and identified some degradation products, which allowed them to suggest that an intracellular PAH transformation pathway was employed by strain SYJ-1.

Further, the versatility of the assembly approach was also confirmed by adding different nanomaterials (TiO2, δ-MnO_2 and α-MnO_2) and using them to remove phenanthrene, which was successful.

Most of the studies carried out on hydrocarbon degradation by *Penicillium* spp. showed that pilot scale and toxicity studies on the metabolites are scarce, being an important point for the design of a suitable wastewater treatment.

Lipids

Fats and oils are the major wastes generated by food processing industries, dairy industries, kitchen activities, bakeries and beverages industries, etc.[21]. In most countries, waste grease has been dumped in the litter site or sewage without any pretreatment leading to severe environmental issues[22]. Grease waste in effluents can cause serious problems such as a reduction in the cell-aqueous phase transfer rates (as well as gas-liquid), reduced sedimentation, and formation of floating sludge, clogging and the emergence of unpleasant odours[80]. For these reasons and due to the high pollutant content of these effluents, it is essential to apply an efficient treatment to release it into the environment. A good option for the treatment of fat-rich wastewater is enzymatic hydrolysis with lipases (Triacylglycerol acylhydrolases, E.C. 3.1.1.3)[21,22,81]. These enzymes catalyze esterification, inter-esterification, acidolysis, alcoholysis and aminolysis in addition to the hydrolytic activity on triglycerides[82] and are largely produced by filamentous fungi like *Penicillium chrysogenum*, *Penicillium cyclopium*, *Penicillium simplicissiimum*, *Penicillium expansum*, *Candida rugosa*, *Aspergillus*, *Trichoderma* etc.[83–85]. For example, Kumar et al.[21] demonstrated the production of a novel lipase by *Penicillium chrysogenum* when it was growing in solid media containing waste grease. This enzyme was isolated, purified, characterized and it was applied on cooking oil waste showing high acid value (26.92 mg·g^{-1}), indicating the presence of free fatty acids.

Later, Kumari et al.[22], reported an effective way to bioremediate grease waste with the combination of lipase pretreatment (commercial lipases from different fungi) and *P. chrysogenum* fermentation. First, the authors pre-treated the grease waste using various lipases and then, this pre-treated grease was used as a substrate by *P. chrysogenum*. The resulting fermented media was analysed and the production of fatty acids was detected, showing high amounts of palmitic acid (2.8 g of palmitic acid recovered from 1.0 kg grease waste). In this case not only bioremediation was successful, but also fatty acid, a value-added product, was obtained from the waste.

Moreover, the treatment of dairy wastewater has been described, using sequential and simultaneous treatment processes, where enzymatic hydrolysis was carried out by an isolate of *Penicillium citrinum*, followed by anaerobic digestion[81]. Free and immobilized whole cells were used as catalysts for the treatment of dairy wastewater. Free whole cells achieved a 1.3-fold higher percent hydrolysis (92.5%) than immobilized whole cells. The biodegradability tests were conducted using crude wastewater, wastewater prehydrolyzed by whole cells, and wastewater simultaneously submitted to whole-cell hydrolysis and biodigestion. The organic matter removal reaches about 43% in all tests. However, the use of whole cells reduced the lag phase time of methanogenic archaea, which accelerated anaerobic digestion, with a higher methane production rate. These results, demonstrated the advantages of using enzymatic hydrolysis combined with anaerobic digestion, whether sequentially or simultaneously.

Other organic pollutants

So far we have reviewed large groups of organic pollutants, of which there are many references as we can see above, dyes, phenols, hydrocarbons, and others. *Penicillium* species have demonstrated their ability to degrade other xenobiotic compounds (Table 4).

In 2014, Luo et al.[86] reported a formaldehyde-degrading *Penicillium chrysogenum* DY-F2 strain, which was isolated from deep sea sediment. This characteristic is interesting, as this makes this fungus useful to be used for the bioremediation of polluted marine environment or wastewater with high salt content. In most studies reported previously, the fungi were isolated from contaminated soils, river sediments or from wastewater treatment plants. *P. chrysogenum* DY-F2 showed

Table 4. Degradation of other organic pollutants by *Penicillium* spp.

Compound	*Penicillium* spp	Reference
Formaldehyde	*P. chrysogenum* DY-F2	[86]
Diethylketone	*Penicillium* spp.	[87]
Polychlorinated biphenyls	*P. chrysogenum*, *P. citreosulfuratum*, *P. canescens.*	[88]
Sodium dodecylbenzene sulfonate	*P. chrysogenum*	[11]
Poly ε-caprolactone and Polyester vylon 200	*P. fellutanum* (Lipases)	[89, 90]

high formaldehyde resistance and was able to grow in the presence of formaldehyde up to 3,000 mg·l^{-1}. In addition, it was able to degrade formaldehyde as the sole source of carbon and energy with the formation of formic acid as the intermediate. This study by Luo et al.[86] was the first to report degradation of formaldehyde by marine fungi.

Some *Penicillium* species, like *P. citreonigrum*, *P. oxalicum, P. chrysogenum*, *P. spinulosum*, *P. verruculosum* and *P. variabile* can efficiently degrade diethyl ketone[87], sodium dodecyl benzene sulfonate[11] and grow well in agar media containing paraffin, chitin, cellulose, leather, pectin, skim milk, sunflower oil, and starch[9] (Table 4). However, the disappearance of the substrates was not measured, and therefore it cannot be confirmed that there was degradation or mineralization of these compounds.

Polychlorinated biphenyls (PCBs) were widely used in electrical equipment and in heat transfer fluids. These pollutants are widespread, persistent, deleterious to the environment and very dangerous for humans. Germain et al.[88] recently described the isolation of four native fungal strains with a remarkable biodegradation capacity, greater than 70%. Three of the four isolates belong to the genus *Penicillium*: *P. chrysogenum*, *P. citreosulfuratum* and *P. canescens.* The last one was the only one that reduced the toxicity related to PCBs and their metabolites, significantly.

Lately, Amin et al.[89,90] described the degradation of poly ε-caprolactone (PCL), a biodegradable aliphatic polyester, and of polyester vylon 200 (PV-200), a synthetic non-biodegradable plastic, by lipases from *Penicillium fellutanum.* These lipases exhibited stability over a broad pH spectrum and by incubation with various industrially relevant organic solvents (benzene, hexanol, ether, and acetone). Under optimal operating conditions, lipase catalyzed the degradation of PCL film leading to 66% weight loss and 81% weight loss for PV-200. These results showed that *P. fellutanum* lipase would be a prospective green and ecofriendly biocatalytic system for efficient degradation and depolymerization of polyester for environmental safety.

Conclusions

The removal of xenobiotics from industrial wastewater is of great interest to avoid environmental contamination. Even though biodegradation and bioremediation with fungi have been well studied, they have not yet been successfully implemented.

Penicillium species showed their ability to adapt their metabolism to many different circumstances and these fungi can use different xenobiotics as a carbon source. In this review, many different capabilities to degrade xenobiotic compounds by *Penicillium* species were summarized. This revision detailed some areas where there are few studies (pilot scale, toxicity, immobilization and consortia studies) and others where there is enough information (fungi isolation and degradation studies); however, in both cases the research should be addressed to obtain new tools for the treatment of wastewater that contain xenobiotic compounds.

For the degradation of phenols and their chlorinated derivatives, most of the *Penicillium* species mentioned in this review were able to use phenol as a sole carbon source (with or without shaking) and degrade chlorophenols in the presence of an auxiliary carbon source, like phenol, glucose, acetate, etc. Most of the studies were carried out with *P. simplicissimum* and *P. chrysogenum* and in batch reactors, while only in one work an up-flow column reactor was operated.

In the search for efficient treatments for the degradation of textile effluents, many studies on dye degradation by *Penicillium* have been carried out. Most of these are in batch culture, testing a few dyes in simulated wastewater and did not test the final toxicity of the degradation products, which is of great importance taking into account the production of toxic aromatic amines. Besides, in the case of azo dyes, the addition of a carbon source is necessary. It is worth mentioning the case of *P. chrysogenum* and *P. ochrochloron* which were tested on real textile wastewater and showed good results.

At the time of this report, *Penicillium oxalicum* was the only species reported for the degradation of pharmaceutical compounds. This subject area has gained importance as in the last few years, antibiotic pollution has increased considerably. For this reason, more studies on this issue have to be carried out.

There are several reports about the biodegradation of PAHs by *Penicillium* species. These studies range from degradation of aromatic hydrocarbons to aliphatic hydrocarbons. Most of the studies showed an increase in degradation by the addition of an external carbon source or surfactants and were carried out in batch cultures with the PAHs tested independently. Therefore, more studies have to be carried out on mixtures of PAHs and crude oil.

Degradation of fats and oils using enzymatic hydrolysis with lipases from *Penicillium* species and the fungi have been successful and also allowed the recovery of fatty acids as a value-added product. In general, *Penicillium* showed good characteristics to be applied in fats and oils treatment, since it could form pellets and can be immobilized on loofa to increase the adsorption and degradation of fats.

In all the studies, no toxicity assays were carried out or only were done on plants and bacteria. The analysis of the toxicity on different species (more than one toxicity test) is very important to understand the efficiency of the biodegradation treatment and select the final destination of the effluent more appropriately, that is, to determine if it can be dumped into the sea or re-used for irrigation, etc. In addition, there is a lack of studies on pilot and full-scale operation processes to solve large-scale problems. The same happens with consortia studies, since taking into account the great ability of different strains of *Penicillium,* one could think of using a consortium made up of several *Penicillium* species with different degrading capacities.

Finally, *Penicillium* strains have proven to be versatile and capable of being used for the biodegradation of different pollutants in wastewater. These fungi can be found in

abundance naturally in the environment and it would be a reasonably cheap solution. However, for all the cases mentioned and summarized in this review, it is clear that beyond the degradation and optimization processes; pilot scale studies and toxicity studies must be carried out to be able to apply these processes for wastewater or bioremediation treatments.

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Conflict of interest

The author declares that there is no conflict of interest.

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