Fungal biodegradation of anthracene-polluted cork - a comparative study

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Abstract

The efficiency of cork waste in adsorbing aqueous polycyclic aromatic hydrocarbons (PAHs) has been previously reported. Biodegradation of contaminated cork using filamentous fungi could be a good alternative for detoxifying cork to facilitate its final processing. For this purpose, the degradation efficiency of anthracene by three ligninolytic white-rot fungi (*Phanerochaete chrysosporium*, *Irpex lacteus* and *Pleurotus ostreatus*) and three non-ligninolytic fungi which are found in the cork itself (*Aspergillus niger*, *Penicillium simplicissimum* and *Mucor racemosus*) are compared. Anthracene degradation by all fungi was examined in solid-phase cultures after 0, 16, 30 and 61 days. The degradation products of anthracene by *P. simplicissimum* and *I. lacteus* were also identified by GC-MS and a metabolic pathway was proposed for *P. simplicissimum*.

Results shown that all the fungi tested degraded anthracene. After 61 days of incubation approximately 86%, 40% and 38% of the initial concentration of anthracene (i.e. 100 µM) was degraded by *P. simplicissimum*, *P. chrysosporium* and *I. lacteus*, respectively. The rest of the fungi degraded anthracene to a lesser extent (< 30%). As a final remark, the results obtained in this study indicate that *P. simplicissimum*, a non-ligninolytic fungi characteristic of cork itself, could be used as an efficient degrader of PAH-contaminated cork.

Keywords: *Quercus suber* L., bioremediation, biodegradation, anthracene, ligninolytic fungi, *Penicillium simplicissimum.*

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of toxic pollutants potentially hazardous to human health because of their carcinogenic, teratogenic and mutagenic character [1,2]. Moreover, these hydrophobic compounds persist in the ecosystems due to their low water solubility and its association with organic matter in soils and sediments [3,4]. For these reasons, the European Union has established for these compounds very restrictive limits in different kinds of surface waters in the proposal Directive 2006/0129 EC which was approved in 2007/C 97/02 [5].

Cork is the bark of the cork oak tree (*Quercus suber* L.), a renewable and biodegradable raw material produced mainly in the Mediterranean region. The main use of cork is for wine bottle stoppers. The cork stoppers industry generates high amounts of by-products of several particle sizes. The annual production of cork waste is around 50,000 tons, which corresponds to an average of 25–30 % of the quantity used in natural cork stopper manufacturing.

Although the most common use of the by-products from the cork industry is combustion for energy production, recent studies have demonstrated their high potential capacity for adsorbing PAH-contaminated water [6,7]. After this removal process, the remaining PAH-polluted cork should be treated to facilitate its final processing and to be reused as for example to obtain other cork products like thermal insulation in refrigerators, shoes or packaging [8].

Microbiological degradation using various types of organisms is the most frequent process used in the decontamination of surface soil and detoxification hazardous waste. Although in general, PAHs are resistant to most standard bioremediation techniques, they can be oxidized to a certain extend by different bacteria and fungi [9,10].

Many biodegradation studies have shown that filamentous fungi are able to metabolize PAHs even more effectively than some bacteria [11,12], so that they could be a good alternative for detoxifying PAH-polluted cork. Among fungi, there are several ligninolytic basidiomycetes that have been tested for anthracene degradation: *Bjerkandera* sp., *Phanerochaete chrysosporium*, *Pleurotus ostreatus*, *Trametes* sp., and *Irpex lacteus* [13-17].

Despite several reports having demonstrated the enormous potential of white-rot fungi such as *P. chrysosporium*, *I. lacteus* and *P. ostreatus* in the biodegradation of PAHs, less is known about the degradation capacity of non-ligninolytic filamentous fungi such as those found in cork itself and which could be considered as an excellent alternative for the biodegradation of PAH-contaminated cork.

The biochemical pathway of PAHs by microorganisms has been described by Gray and Thorton [18]. Two mechanisms are described to be involved in PAH degradation by fungi: one is based on the action of the intracellular cytochrome P450 monooxygenases, generating epoxides and dihydrodiols intermediates; the other is carried out by extracellular ligninolytic enzymes (i.e. lignin peroxidase, manganese dependent peroxidase and laccase) producing cation radicals from contaminants followed by the formation of quinones [19-24]. Due to the fact that the intermediate metabolites generated in PAHs oxidation might be more toxic than the initial compounds, it is important to identify the compounds generated and select the proper degrading microbes.

Exist different microorganisms associated with cork samples during the different manufacturing processes of cork stoppers (i.e. boiled of the planks, cut and punched into cylindrical pieces and cleaned and surface treated with paraffin or/and silicon to obtain the final product). Filamentous fungi such as *A. niger*, *P. simplicissimum* and

Mucor racemosus have been previously found at the final step of the production of cork stoppers [25] and have been selected in this study because of their ability to remove some xenobiotic compounds [19, 26-32].

For the cork industry, the development of a new biotechnology based on the use of cork by-products would revalue this residue and would be an incentive for future forest management of this vegetable material. The decontamination of cork adsorbent by using the characteristic fungi of cork itself is an essential step towards understanding its viability and future potential use.

Thus, the aims of this study are (1) to investigate and compare the ability of three ligninolytic fungi (*P. chrysosporium*, *I. lacteus* and *P. ostreatus*) and three non-ligninolytic fungi characteristic of cork itself (*A. niger*, *P. simplicissimum* and *M. racemosus*) to degrade anthracene from contaminated cork samples, (2) to identify the anthracene degradation products generated and (3) to elucidate the degradation pathway in the fungus that shows the highest anthracene degradation.

Material and methods

Chemicals and reagents

Anthracene, 4-terphenyl-d_{14}, anthracene-d_{10} and phenanthrene-d_{10} were obtained from Supelco (Bellefonte, USA). Phthalide, dimethyl phthalate, phthalic anhydride and anthrone standards were supplied by Sigma-Aldrich. The ethyl acetate used was super purity grade from Romil (Cambridge). Stock standard solutions of these chemicals were prepared in ethyl acetate and stored in glass bottles in the dark at 4ºC. Sodium sulphate was purchased by Panreac and Fluorisil by Sigma-Aldrich.

Organisms and culture conditions

A. niger (CECT 2545), *M. racemosus* (CECT 2670) and *P. simplicissimum* (CECT 20706) belong to the Spanish Collection of type cultures (CECT). *P. ostreatus* (IJFM A579), *P. chrysosporium* (IFKM A547) and *I. lacteus* (IJFM A792) were collected from the Fungal Culture Collection of the Centro de Investigaciones Biológicas (Madrid, Spain).

The fungal strains were grown in 1L erlenmeyer flasks in 250 mL of modified Czapeck-Dox medium prepared with 1% glucose, 0.2% ammonium tartrate and 0.1% yeast extract in shaken conditions (220 rpm) and controlled temperature (28ºC). Inocula were prepared by homogenizing the mycelia after 10 days of incubation.

Contaminated cork samples preparation

Samples of cork waste from Catalonia were cut into small pieces (< 10 mm) and milled in a ZM-200 ultra centrifugal mill (Retsch, Netherlands) and the granulometric fraction of 40 to 60 mesh (0.25 to 0.42 mm of particle size) was subsequently used. Cork waste sample selected for this study (CAT_1) has been previously characterized [33] .

Series of 2 g of granulated cork obtained previously was placed inside an erlenmeyer with 50 ml of anthracene solution at 100 µM (0.46 mg g^{-1}). All recipients were mixed with a Vibromatic oscillating shaker at 700 oscillations min^{-1} during 1 hour. Before inoculation, samples were dried at ambient temperature during 48 hours.

Solid-state fermentation cultures

2 g of cork contaminated with anthracene were inoculated with 6 ml of each homogenized fungal mycellium. The solid-state fermentation (SSF) cultures were

incubated for different periods (0, 16, 30 and 61 days). In addition, a flask with contaminated cork and without fungi was prepared as abiotic control to monitor non-biological losses during all the experiment. All flasks were wrapped with aluminium foil to prevent photodegradation and were maintained under controlled conditions (28ºC and 60% humidity) during all time-course assays. Finally the bio-treated cork samples were filtered and frozen until analysis. All the experiments were performed in triplicate.

Anthracene extraction

Defrosted samples were placed in an extraction thimble (Whatman cellulose) and 4-terphenyl d_{14} (0.1 mg g^{-1}) was added as standard of the extraction. Soxhlet procedure using ethyl acetate for 7 h was performed. After this extraction time, solvent was evaporated to dryness in a rotary evaporator (Aircontrol, Spain) at 40ºC. This residue was dissolved in 10 ml of ethyl acetate using an ultrasonic bath (Banderlin Sonorex, Germany) and was subsequently dried over Na_2SO_4 and Fluorisil and evaporated to dryness. The final extract was dissolved in 10 ml of ethyl acetate using ultrasonic bath.

Analysis of anthracene

Anthracene was measured using a gas chromatograph (GC) (Agilent 6890 180 N) coupled to a mass spectrometer (MS) (Agilent 5973N). The separation was achieved using an HP-5MS column (30 m length, 0.25 mm I.D., 0.25 μm film thickness) (J&W Scientific, Folsom, CA, USA). A volume of 1 µl of the sample solution was injected in a splitless mode. GC oven program started at 60ºC (1 min), increased by 25ºC min^{-1} to 150 ºC, 10ºC min^{-1} to 260ºC (held for 20 min) and increased to 270ºC (held for 20 min). The carrier gas was helium (99.999 %) from Abello Linde (Barcelona, Spain) with a constant flow rate of 1 mL min^{-1}. Internal standard calibration using phenanthrene d_{10}

was used for anthracene quantification. The mass spectrometer was operated in SIM mode detecting the following ion masses: 178, 188 and 244. All analysis were performed in triplicate.

Analysis of anthracene degradation products

Anthracene degradation products were analyzed by GC coupled to MS on an integrated quadrupole MD-800 (Thermo, Manchester UK). The GC separation was performed on a DB-5 column (60 m length, 0.25 mm I.D., 0.25 μm film thickness) from J&W (Folsom, CA, USA). Splitless injection mode was used for 1 min and injection volume was 1 µL. Oven temperature program was from 70ºC held 1 min to 310ºC maintained 1 min at 7ºC min^{-1}. Injector and interphase temperature were 270ºC and 280ºC, respectively. Helium was used as carrier gas at a constant head pressure of 100 kPa. Mass spectrometry was performed using the electron ionization mode (EI+) at 70 eV of ionization energy. Ion source temperature was 250ºC. Acquisition was carried out in the full scan mode from m/z 45 to 450 at 0.5 s $scan^{-1}$ with an interscan time of 0.1 s. Analytes were quantified from their response factor related to anthracene-d_{10}, used as internal standard. Blanks with hexane and without sample were also analyzed to assess the possible contamination of samples by phthalates from plastic during preparation, storage and analysis. All analysis were performed in duplicate.

Identification of anthracene degradation products were confirmed using standards of metabolites (2-anthrone, 9,10 anthraquinone, phthalide acid, and phthalide) as shown in Fig.1.

Results and discussion

Anthracene biodegradation

The percentages of anthracene removal from contaminated cork after 16, 30 and 61 days of solid-state fermentation (SSF) with the different fungi are shown in Fig.2. To calculate the biodegradation percentages, SSF samples of 0 day-incubation period were used as reference for the initial PAH concentration. The abiotic control showed anthracene losses less than 1% (data not shown).

As shown in Fig.2, all fungi were able to remove anthracene from cork after 61 days of incubation, although with rather different yields. *P. simplicissimum* showed the highest percentage of anthracene degradation (86%). In contrast, *P. ostreatus* demonstrated the lowest percentage of anthracene degradation (15%). The final percentages of anthracene removal obtained with the other white-rot fungi, *P. chrysosporium* and *I. lacteus*, were higher (~40%) than those obtained with the non-ligninolytic fungi *A. niger* (31%) or *M. racemosus* (24%).

Fig.3 shows the degradation of the anthracene peak by *P. simplicissimum* over time. It was calculated that 42% of anthracene degradation was produced during the first sixteen days.

These results are consistent with the fungal growth observed on the contaminated cork. *P. ostreatus* showed the poorest colonization of the substrate compared to the control (without fungi). In contrast, *P. simplicissimum* grew rapidly and the mycelium covered the whole cork surface after 16 days of incubation (Fig. 4). The rest of the fungi exhibited moderate growth.

In general, anthracene removal was observed to increase along with the increase in time period of solid-state fermentation (SSF), except in the cases of the degradation percentages attained with *A. niger* and *P. ostreatus* which remained fairly constant after the first 16 days of incubation (Fig. 2).

Table 1 summarizes the data reviewed from literature on anthracene degradation yields produced by filamentous and non-filamentous fungi. Although it is difficult to compare because of the different parameters used some general aspects are worth mentioning. The degradation values ranged from 20% up to the complete removal of anthracene. Moreover, a great variability in the percentages of anthracene degradation within each ligninolytic fungi was observed. These variations depended on various factors such as the initial concentration of the target pollutant or the culture conditions such as type of medium, pH or temperature and the bioavailability of the pollutant. It was also observed that the percentage of PAH biodegradation was significantly higher in liquid than in solid culture conditions, probably due to the decreased PAH availability in solid medium [34,35]. Even so, there are some studies on solid cultures that demonstrate higher degradation rates (Table 1), probably due to the effect of the different factors mentioned above [14, 17, 36].

With ligninolytic fungi, similar percentages of anthracene degradation were obtained by *I. lacteus* after 120 days of incubation in creosote contaminated soil with a 32-38% PAH mix [15], and in sterilized soil after 70 days [17]. On the contrary, PAH degradation studies with *P. chrysosporium* and *P. ostreatus* in a solid medium, reported percentages of anthracene removal significantly higher (over 90%) [14, 36], to those obtained in this study (around 40 and 15%, respectively). Note that, the high percentages of anthracene removal reported in the literature for *P. chrysosporium* were performed at higher initial anthracene concentrations (0.05-1 g L^{-1}) compared to that used in this study (0.017 g L^{-1}) [13, 14, 37]. Bhatt et al. [38] and Byss et al. [15] described the effect of the initial PAH concentration on anthracene biodegradation. However, while Bhatt et al. [38] had reported increments of 20-30% in anthracene degradation when the initial concentration of the PAH was duplicated, Byss et al. [15] did not find any significant differences in the PAH removal by *P. ostreatus* and *I. lacteus* when the initial concentration was increased from 0.7 mg kg^{-1} to 32 mg kg^{-1}.

It is worth noting the high anthracene degradation efficiency found in this study for *P. simplicissimum* (86%), and which were similar to the above mentioned values found in the literature for the three selected ligninolytic fungi (Table 1). Similar anthracene degradation values (around 30 %) to those obtained in this study (29%) have been reported for *Aspergillus niger* [39]. However, to the best of our knowledge, this study is the first in which anthracene degradation for the non-ligninolytic fungi *Penicillium simplicissimum* and *Mucor racemosus* is described.

The degradation studies with high molecular weight PAHs for non-ligninolytic fungi revealed that Zygomycetes, and in particular *M. racemosus*, appeared as one of the most efficient taxonomic groups to degrade these types of PAHs (4-7 rings) such as pyrene and benzo[a]pyrene [11,40]. *P. simplicissimum* and *M. racemosus* have also demonstrated the ability to remove pyrene in a liquid synthetic medium after 2 days (2.4 and 3.26 mg g^{-1} of pyrene, respectively) [19,30]. Since the above-mentioned PAHs are more recalcitrant to biodegradation than low molecular PAHs, the capability of the non-ligninolytic fungi *P. simplicissimum, M. racemosus* and *A. niger* to remove, for instance anthracene, could be inferred; as was demonstrated in this study under SSF conditions.

Given that *P. simplicissimum* is a fungus characteristic of cork [41], the outstanding capability of this fungi to remove anthracene from contaminated cork, demonstrated here, represents a valuable step forward for the future development of a bio-technique to detoxify contaminated cork. Therefore, the intermediate oxidation products were analyzed to assess the degradation pathway used by *P. simplicissimum* and whether the toxic compound was transformed onto harmless substances or not. For the sake of comparison, the intermediates generated during the oxidation of anthracene with the ligninolytic fungi *I. lacteus* were also identified.

Metabolic products from the biodegradation of anthracene by P. simplicissimum and I. lacteus

Following the biotreatment of contaminated cork with *P. simplicissimum* and *I. lacteus*, after 0, 16 and 61 days of incubation, the anthracene degradation products obtained were identified using GC-MS. The retention times, mass spectral characteristics of anthracene and its metabolites analyzed in all cork extracts are shown in Table 2.

Anthraquinone, anthrone and traces of phthalic acid and phthalic anhydride were found in the final extracts of *P. simplicissimum* and *I. lacteus*. In contrast, compounds such as 9-anthrol, 2-(2'-hydroxybenzoyl)-benzoic acid and anthracene trans-1,2-dihydrodiol were not found in any extract from the respective periods.

While the maximum abundance of anthrone, which was 0.16% and 0.15% of the initial concentration of anthracene for *I. lacteus* and *P. simplicissimum* cultures, respectively, was detected after 16 days, it was not detected at the end of the SSF (61 days) with either fungus. 9-10-anthraquinone was found to be the principal oxidation product detected in the 16-day samples. It accumulated in the both fungal cultures after 16 days

(8.5% and 1.2% for *I. lacteus* and *P. simplicissimum,* respectively) and decreased after 61 days (5.3% and 0.07% for *I. lacteus* and *P. simplicissimum*, respectively). This suggests that, although 9-10-anthraquinone is a stable intermediate [42], it is not the end oxidation product [43]. This was confirmed by the detection of traces of phthalic acid and phthalic anhydride (0.58% and 0.09% for *I. lacteus* and *P. simplicissimum*, respectively) after 16 days of SSF. These results demonstrate the fungal capability to perform further degradation steps until cleavage of the aromatic ring.

In line with these intermediate products detected, Fig.5 shows the proposed pathway for anthracene degradation by *P. simplicissimum*. This degradation pathway has previously been reported for ligninolytic fungi [11,42,44-47] and non-ligninolytic fungi [48]. Accumulation of PAH-quinones, is characteristic of PAH degradation by white-rot fungi under ligninolytic conditions [11,13,49,50], and constitutes a good detoxification alternative as quinones are less toxic [51,52] and more bioavailable than the initial PAH and subsequently they may be readily degraded by bacteria [53]. In this study, production of 9,10-anthraquinone during the degradation of anthracene by *P. simplicissimum* (although in a significantly lower amount than with I. lacteus) suggests the involvement of some ligninolytic oxidoreductase activity (laccase or peroxidase) in this process. According to GenBank and UniProt databases, laccase and peroxidase activity has been also described in other *Penicillium* sp. such as *P. roqueforti*, *P. digitatum*, *P. oxalicum*, *P. chrysogenum, P. citrinum, P. expansum* and only laccase activity has been detected in *P. marnefrei and P. stipiatatus*

The presence of anthrone in the final extract of non-ligninolytic fungi evidences the possible involvement of the extracellular ligninolytic system in anthracene degradation by *Penicillium simplicissimum* [13,54]. Clemente et al.[55] and Zeng et al.[31] also detected ligninolytic activity during the growth of non-ligninolytic fungi in PAH. In the case of *P. simplicissimum*, ligninolytic activity has been shown to mainly occur during the primary metabolism [56]. The presence of ligninolytic enzymes in non-ligninolytic fungi such as *Trichoderma reesei* and *Fusarium proliferation* has also been elucidated [57,58].

Conclusions

Results obtained in this work have shown that, to a greater or lesser degree, white-rot fungi (i.e. *Phanerochaete chrysosporium*, *Irpex lacteus* and *Pleurotus ostreatus*) and ascomycete fungi characteristics of cork (i.e. *Aspergillus niger*, *Penicillium simplicissimum* and *Mucor racemosus*) are able to degrade anthracene in anthracene-polluted cork. After 61 days of incubation, *P. simplicissimum* produced the highest degree of anthracene removal (86%) followed by *P. chrysosporium*, *I. lacteus* (40%), *A. niger* (31%), *M. racemosus* (24%) and *P. ostreatus* (15%).

Anthraquinone, anthrone and traces of phthalic acid and phthalic anhydride were detected as intermediate products from anthracene degradation by *P. simplicissimum* and *I. lacteus*. According to this, the following degradation pathway for *P. simplicissimum* was proposed: initially, anthracene was oxidized to anthrone, which was then further transformed to anthraquinone, and finally, trace levels of phthalic acid were produced by ring cleavage. The proposed pathway for anthracene degradation by *P. simplicissimum* is similar to that previously described for ligninolytic fungi. This fact is beneficial for the main purpose of this study, i.e. the detoxification of PAH-contaminated cork, because anthraquinone is less contaminating and more easily biodegradable than anthracene

Thus, the biodegradation capacity and detoxification pathway obtained from anthracene, suggest than *P. simplicissimum* could be used efficiently to bio-remediate PAHs polluted cork.

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FIGURE CAPTIONS

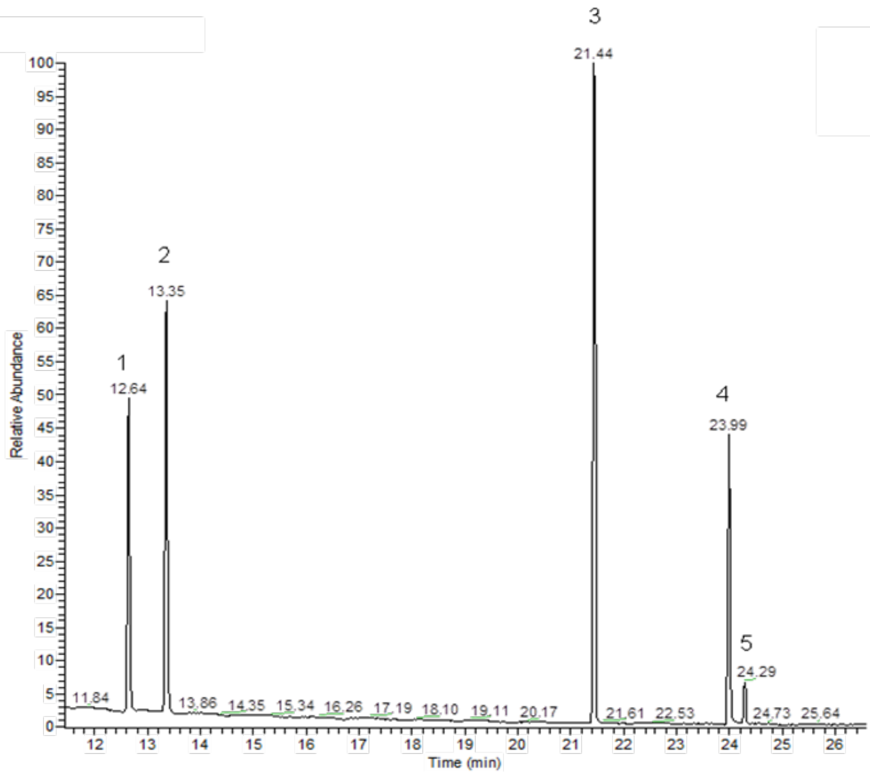
Figure 1.- Chromatogram of standards of the metabolites analyzed from anthracene degradation (1-anthracene. 2-anthrone, 3- 9,10 anthraquinone, 4-phthalide acid, and 5-phthalide).

Figure 2. Removal of anthracene from contaminated cork by solid-state fermentation with three non-ligninolytic fungi (Ps: *Penicillium simplicissimum*; An: *Aspergillus niger*; Mr: *Mucor racemosus*) and three ligninolytic fungi (Il: *Irpex lacteus*; Pc: *Phanerochaete chrysosporium*; Po: *Pleurotus ostreatus*) after 16, 30 and 61 days of incubation. Bars are standard deviations of triplicate analysis.

Figure 3. Chromatogram of the peak of anthracene degradation by *P. simplicissimum* after 0 (black), 16 (red) and 61 (green) days of incubation.

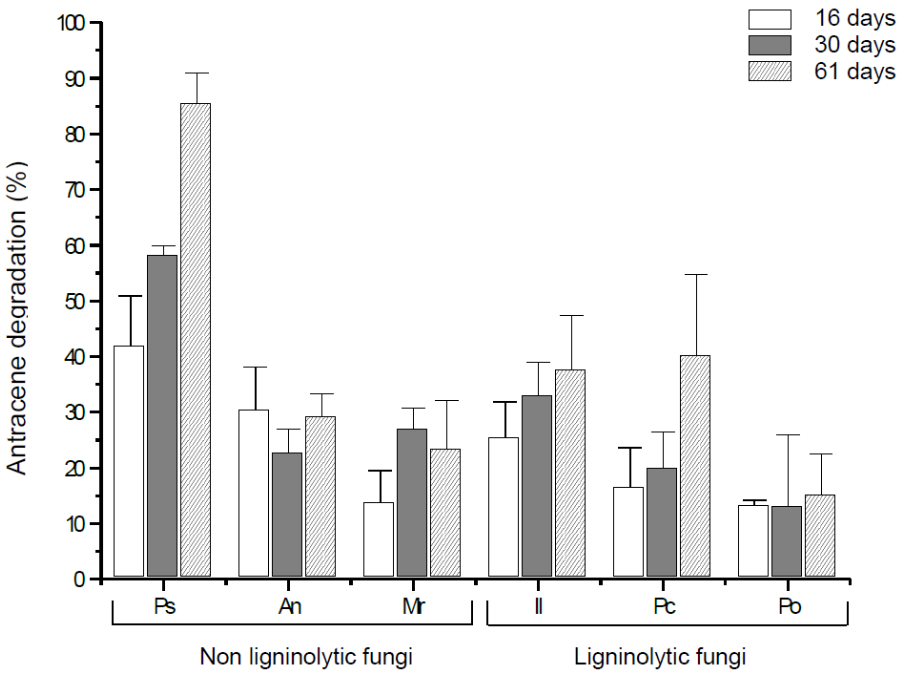
Figure 4. Abiotic control (i.e. without fungus) (C) and fungal growth (Po: *Pleurotus ostreatus*; Ps: *Penicillium simplicissimum*) in contaminated cork after 61 days of incubation.

Figure 5.- Proposed pathway of anthracene degradation by *Penicillium simplicissimum.*



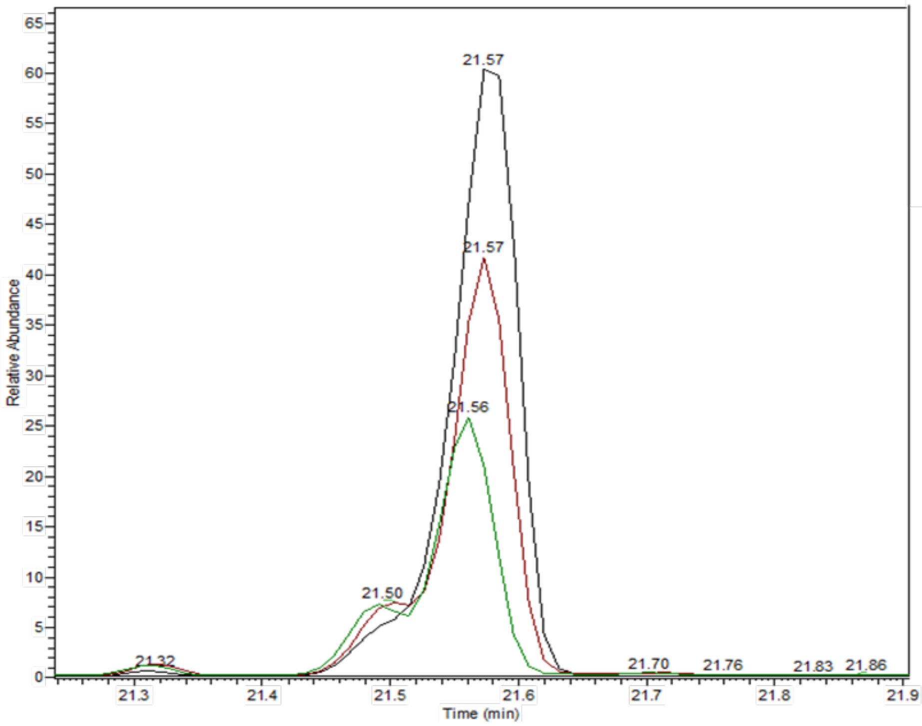
3
21.44
2
13.35
1
12.64
4
23.99
5
24.29
11.84
13.86
14.35
15.34
16.26
17.19
18.10
19.11
20.17
21.61
22.53
24.73
25.64
Relative Abundance
Time (min)

Fig. 1



16 days
30 days
61 days
Antracene degradation (%)
100
90
80
70
60
50
40
30
20
10
0
Ps
An
Mr
Il
Pc
Po
Non ligninolytic fungi
Ligninolytic fungi

Fig. 2



65
60
55
50
45
40
35
30
25
20
15
10
5
0
Relative Abundance
21.57
21.57
21.56
21.50
21.32
21.70
21.76
21.83
21.86
21.3
21.4
21.5
21.6
21.7
21.8
21.9
Time (min)

Fig. 3

Fig. 4

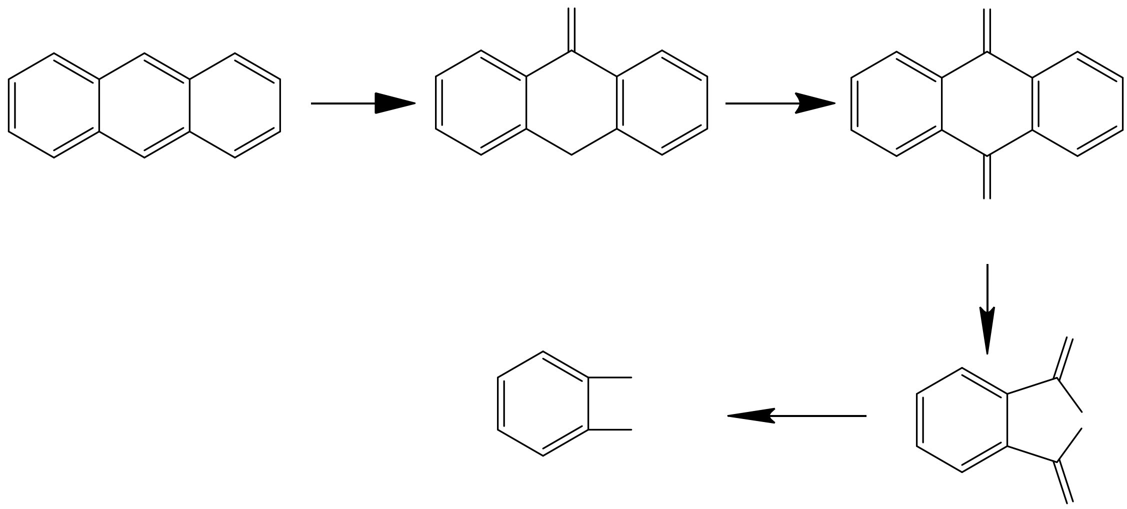


Fig. 5

Table 1. Comparison of anthracene degradation by several fungi reported in the literature.

Organism	Growth substract	Initial anthracene concentration	pH	T (ºC)	Incubation time (days)	% removal	Reference
Irpex lacteus	Contaminated cork	0.017 g/L	-	28	61	37.8	This study
	Liquid culture	0.025 g/L	-	28	14	60	Novotný et al. [16]
	Tyndallized dry soil	0.05 g/L	-	26	90	49	Novotny et al. [16]
	Liquid culture medium	0.025 g/L	-	-	50	>95	Cajhaml et al. [42]
	Liquid culture medium	0.025 g/L	-	-	50	77.7-99.2	Cajhaml et al. [21]
	Polluted soil	0.7 mg/Kg	-	15	120	32	Byss et al. [15]
		32 mg/Kg	-	15	120	38	
	Sterilized soil	18.8 mg/Kg	-	-	35	60.6	Borràs et al. [17]
		14.2 mg/Kg			70	42.9	
	Unsterilized soil	18.8 mg/Kg			35	47.3	Borràs et al. [17]
		14.2 mg/Kg			70	47.8	
	Industrial soil	229 mg/kg	6.49	26	98	72.9	Bhatt et al. [38]
		99 mg/kg	6.85	26	98	47.8	
	Sterilized quartz sand	1.6 g/L	-	28	42	69.4	Leonardi et al. [2]
Phanerochaete chrysosporium	Contaminated cork	0.017 g/L	-	28	61	40.2	This study
	Liquid culture medium with anthracene oil	0.05 g/L	-	39	27	98	Bumpus [37]
	Liquid culture medium	1 g/L	-	-	28	99.2	Field et al. [13]
	Liquid culture medium	0.01 g/L	-	37	4	58	Krivobok et al. [29]
	Sterilized soil	5 mg/Kg	7	30	42	92.6	Bishnoi et al. [14]
Pleurotus ostreatus	Contaminated cork	0.017 g/L	-	28	61	15.2	This study
	Creosote contaminated soil	0.7 mg/Kg	-	15	120	64	Byss et al. [15]
		32 mg/Kg				77	
	Liquid culture medium	0.5 g/L	-	22	12	62	Schützendübet et al. [50]
	Liquid culture medium	8.3 g/L	-	28	21	74	Bezalel et al. [44]
	Polluted soil	220 mg/kg	-	-	84	50	Eggen and Sasek [9]
	Sterilized quartz sand	1.6 g/L	-	28	42	78	Leonardi et al. [2]
	Industrial soil	229 mg/kg	6.49	26	98	70.3	Bhatt, et. al. [38]
		99 mg/kg	6.85	26	98	32.6	
	Drill cuttings	10.92 mg/Kg	-	30	56	100	Okparanma et al. [36]
Aspergillus niger	Contaminated cork	0.017 g/L		28	61	29.4	This study
	Liquid culture medium	0.01 g/L	-	22-24	4	27.1-37.5	Giraud et al. [39]
Penicillium simplicissimum	Contaminated cork	0.017 g/L	-	28	61	85.5	This study
Mucor racemosus	Contaminated cork	0.017 g/L	-	28	61	23.5	This study

Table 2. - Retention data and mass spectral characteristics of anthracene and its metabolites analyzed in all cork extracts.

Product no.	t_R (min)	m/z ions (relative intensity)	Structural suggestion
1	21.44	178 (100), 179 (15.7), 176 (14.1), 89 (7.6)	anthracene
2	23.99	194 (100), 165 (98.4), 138 (49.6), 81 (37.1)	Anthrone
3	24.29	208 (100), 180 (64.2), 152 (58.8), 126 (4.4), 76 (5.9)	9,10-anthraquinone
4 and 5	12.64	104 (100), 148 (18.7)	Phthalic acid and phthalic anhydride
6	-*	166 (100), 194 (38.0)	Anthracene trans-1,2-dihydrodiol
7	-*	224 (100), 196 (41.0), 168 (32.8), 242 (14.4), 139 (14.1)	2-(2’-Hydroxybenzoyl)-benzoic acid
8	13.35	105 (100), 77 (40.9), 134 (12.7), 51 (9.0)	phthalide

* Analytes identified by m/z ions and relative intensities