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Dechlorination of 1,2,3- and 1,2,4-trichlorobenzene by the white-rot fungus *Trametes versicolor*

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ABSTRACT

The degradation of 1,2,3-, 1,3,5- and 1,2,4-trichlorobenzene (TCB) by the white-rot fungus *Trametes versicolor* was studied. Time course experiments showed a degradation rate of 2.27 and 2.49 nmol d^{-1} mg^{-1} dry weight of biomass during the first 4 d of incubation in cultures spiked with 6 mg L^{-1} of 1,2,3- and 1,2,4-TCB, respectively. A high percent of degradation of 91.1% (1,2,3-TCB) and 79.6 (1,2,4-TCB) was obtained after 7 d. However, *T. versicolor* was not able to degrade 1,3,5-TCB under the conditions tested. For a range of concentrations of 1,2,4-TCB between 6.5 and 23 mg L^{-1}, a complete dechlorination of the molecule was observed. Cytochrome P450 monooxygenase appears to be involve in the first step of 1,2,4-TCB degradation, as evidenced by marked inhibition of both dechlorination and degradation of 1,2,4-TCB in the presence of the known cyt P450 inhibitors 1-aminobenzotriazole and piperonyl butoxide. Four intermediates formed from 1,2,4-TCB degradation were detected the second day of incubation, which did not appear the seventh day: 2,3,5-trichloromuconate, its corresponding carboxymethylenebutenolide, 2- or 5-chloro-4-oxo-2-hexendioic acid and 2- or 5-chloro-5-hydroxy-4-oxo-2-pentenoic acid. Based on these results, a degradation pathway of 1,2,4-TCB through cyt P450 monooxygenase and epoxide hydrolase was proposed.

1. Introduction

Chlorobenzenes (CBs) are widely used for industrial and domestic purposes such as solvents, degreasers, pesticides and as intermediate in the production of other chemicals [1,2]. Due to the persistence, toxicity and the bioaccumulation potential of CBs, biodegradation has been proposed as a feasible technology to treat soils and waters contaminated by these organic pollutants [3–5].

Aerobic microbial degradation is well documented for mono- and dichlorobenzenes whereas reports regarding degradation of more highly chlorinated benzenes by pure cultures are more limited [6,7]. Aerobic chorobenzene-degrading bacteria use these pollutants as sole carbon and energy source, producing chlorocatechol as an intermediate by the action of a constitutively expressed chlorobenzene dioxygenase and dihydrodiol dehydrogenase [8,9]. The chlorocatechols formed are further degraded leading to intermediates of central metabolic routes [10].

Reductive dechlorination by different anaerobic microbial communities was also detected [11,12]. Adrian et al. [13] reported the isolation of a bacterium, *Dehalococcoides* sp. strain CBDB1, that is able of coupling its growth to the dechlorination of several chlorobenzenes, including 1,2,3-trichlorobenzene (1,2,3-TCB), 1,2,4-trichlorobenzene (1,2,4-TCB), and all three tetrachlorinated benzenes. In most cases, a stoichiometric conversion to less chlorinated isomers, but no mineralization, was observed [1,14–16].

In comparison to the wealth of information on bacterial degradation of CBs, there is only one report regarding CB degradation by fungi in the literature [17]. In that report, the white-rot fungus *Phanerochaete chrysosporium* was shown to degrade and mineralize monochlorobenzene and different isomers of dichlorobenzene. Interestingly, maximal degradation and mineralization of CBs were found in malt extract cultures in which peroxidases, the most common enzymatic system involved in degradation of pollutants by white-rot fungi, are not known to be produced.

The objective of this work is to investigate the ability of the white-rot fungus *Trametes versicolor* to degrade chlorobenzenes with more than two chlorine substituents, to be exact the three isomers of trichlorobenzenes that show increasing persistence against aerobic biodegradation.

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2. Materials and methods

2.1. Fungal strains and chemicals

T. versicolor (ATCC#42530) was obtained from the American Type Culture Collection. The strain was maintained by subculturing on 2% malt extract agar slants (pH 4.5) at room temperature. Subcultures were routinely made every 30 d.

1,2,4-TCB was obtained from Fluka (≥99%). 1,2,3-TCB (99%) and 1,3,5-TCB (99%) were obtained from Aldrich (Barcelona, Spain). Cytochrome P450 inhibitor 1-aminobenzotriazole (ABT) was obtained from Sigma (Barcelona, Spain) whereas piperonyl butoxide (PB ≥90%) was obtained from Fluka (Barcelona, Spain).

2.2. Composition of the medium and production of T. versicolor pellets

Defined medium contained 8 g L^{-1} glucose, 498 mg L^{-1} N as ammonium tartrate, 10 and 100 mL L^{-1} of micro and macronutrients, respectively [18], and 1.168 g L^{-1} of 2,2-dimethylsuccinate buffer.

Pellets of *T. versicolor* were produced by inoculating 1 mL of a mycelial suspension to 250 mL of malt extract medium in a 1 L Erlenmeyer flask, as described elsewhere [19].

2.3. In vivo degradation experiments of TCBs

All the experiments were performed using 125-mL serum bottles (Wheaton, Millville, NJ) sealed with Teflon-coated grey butyl rubber stoppers (Wheaton, Millville, NJ) and aluminium crimps (Baxter Scientific Products, McGaw Park, IL). Each bottle was inoculated with 2 g of wet pellet of *T. versicolor* (equivalent to 5.0 g L^{-1} dry weight). 10-mL of liquid medium was added in each inoculated bottle and subsequently was oxygenated for 1 min and sealed immediately. Then, 20 μL of TCB in ethanol was added by means of a pressure-lok gas-tight syringe (VICI Precision Sampling, Baton Rouge, LA) through the stoppers to give the required concentration in the liquid media. The bottles were incubated at 25 °C in shaken conditions (135 rpm, r = 25 mm), also in an inverted position.

Each experiment included uninoculated and heat-killed controls. Percent degradation at a specified interval was calculated by comparing concentration in the uninoculated blanks with those in the experimental bottles. All degradation values were corrected for the sorption values determined using the heat-killed controls. TCBs concentration values were also corrected considering the water volume added with pellets. Each bottle was sacrificed at each time point for analysis.

2.4. TCBs and chloride release analysis

First, 0.5 mL of sample was withdrawn with a plastic syringe and subsequently filtered through a Millex-GV (Millipore) 0.22 μm filter. This sample was used to quantify the chloride releases in the medium by means of an ICS-2000 ion chromatography system (Dionex Corp., Sunnyvale, CA, USA), which includes an eluent generator and conductivity detector. Dionex Chromeleon software version 6.8 was used for data processing. Dionex IonPac AS19-HC (4 mm × 250 mm) and AG19-HC (4 mm × 50 mm) columns packed with anion-exchange resin were used as the separation columns. Deionized water was supplied to the suppressor for continuous regeneration of the ion-exchange sites via electrolysis, using 112 mA current. A 25 μL injection was made with a Dionex AS40 autosampler. The analysis was performed at 30 °C with the flow rate set at 1.0 mL min^{-1}. The eluent was generated as potassium hydroxide solution by the eluent generator. The eluent concentration was 10 mM from 0 to 5 min and 10–45 mM from 10 to 25 min.

After this first withdrawn, we proceed to determine the concentration of TCBs by static headspace gas chromatography. All samples were equilibrated at 25 °C before analysis. Thus, 10 mL of acetonitrile were added to each sample bottles and were agitated with an orbital shaker (135 rpm, r = 25 mm) for 20 min. Subsequently, 1 mL liquid sample from each experimental bottle was transferred to 4 mL sodium azide solution (1%) in a 10 mL vial and sealed immediately with a teflon coated stopper. The vial was placed in a headspace sampler Agilent 7964 (Agilent Technologies, Palo Alto, CA) and was heated to 85 °C for 25 min. Subsequently, a 1-mL headspace sample was injected automatically into a gas chromatograph (Agilent 6890N) equipped with a column Agilent HP-5 (30 × 0.32 × 0.25) and a flame ionization detector. The GC operating conditions were as follow: column temperature, 40 °C (2 min), slope 4 °C min^{-1}, 50 °C (1 min), slope 10 °C min^{-1}, final temperature: 200 °C (0.5 min); injector temperature, 125 °C; flame ionization detector temperature, 260 °C; carrier gas, He at 7 psi pressure. Data was acquired and quantified by Millennium 32 software (Waters, Milford, MA).

Total amount of target compounds in the experimental bottles and its concentration in liquid media were determined by comparing peak areas with those of external standards and by using a Henry's law constant value of 0.46 M atm^{-1} (1,2,4-TCB) [20], 0.43 M atm^{-1} (1,2,3-TCB) and 0.63 M atm^{-1} (1,3,5-TCB) [21].

2.5. Experiments with cytochrome P450 inhibitors

For those cultures that were tested with the cytochrome P450 inhibitors ABT or PB (in an ethanol solution), a final concentration of 5 mM of these inhibitors were added to cultures. The sample bottles were performed as was stated above and they were incubated for 9 d. Heat-killed and inhibitor-free controls were also included in this experiment by triplicate.

2.6. Experiments with purified laccase

Possible involvement of laccase in 1,2,4-TCB degradation was investigated using 125-mL serum bottles sealed with Teflon-coated stoppers containing 10 mL of a purified laccase solution (final enzyme activity of 5000 AU L^{-1}) at pH 4.5. 1,2,4-TCB was added at a final concentration of 10 mg L^{-1}. Effect on laccase of different mediators, such as ABTS [2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)], 1-hydroxy-benzotriazole (HOBT), voluric acid (VA), and 3,5-dimethoxy-4-hydroxyacetophenol (DMHAP), were tested at a final concentration of 0.8 mM (except for HOBT which was added at 1 mM) into the reaction mixture. The bottles were incubated on an orbital shaker (135 rpm) at 25 °C for 48 h. A control test containing the same amount of 12,4-TCB in deionized water at pH 4.5 was performed in parallel. Each treatment was carried out in duplicate.

2.7. Identification of 1,2,4-TCB metabolites

In order to identify the degradation products of 1,2,4-TCB, 2 and 7 d old cultures broth, freed from cells by centrifugation, were extracted three times with equal volumes of ethyl acetate. The pH of the medium was 4.5. The organic layers were dried over anhydrous sodium sulphate and after filtration the solvent was evaporated, obtaining the dry sample. Two different controls were included, heat-killed cultures (that were considered the control at time zero) and fungus alive without 1,2,4-TCB. Seven replicates were carried out for each case.

The kinetics of the 1,2,4-TCB degradation by *T. versicolor* was analysed by Nuclear Magnetic Resonance (NMR) Spectroscopy and Electro Spray Ionization Mass Spectroscopy (ESI-MS).

For the NMR analysis each dry sample was dissolved in 500 μL of CH_3OH-d_4. ^1H (600.13 MHz) NMR spectra were acquired on an AVANCE 600 Bruker spectrometer (Bruker Biospin, Karlsruhe, Germany). When necessary, COSY (Correlated Spectroscopy) experiments were performed in order to confirm the 1,2,4-TCB structure. All experiments were recorded at 25 °C and tetramethylsilane was used as external reference.

Direct ESI-MS analyses were performed on an Esquire 6000 octopole ion trap mass spectrometer (Bruker Daltonics, Karlsruhe, Germany). All mass spectra were acquired in the range of m/z 50–500 and in the negative-ion ESI mode. The NMR samples were diluted in 500 μL of CH_3CN (MS grade) and were directly injected into the ESI source. The electro spray conditions were: dry gas (N_2) at 8 L min^{-1}, dry temperature at 300 °C and nebulizer at 40 psi.

2.8. Other analysis

Fungal dry weights and laccase activity were determined as is described before [19]. Manganese and lignin peroxidase (MnP and LiP) activities were determined as described elsewhere [22].

3. Results and discussion

3.1. Degradation of TCBs by T. versicolor

The ability of *T. versicolor* to degrade TCBs in pure culture was determined in a defined medium. Degradation of 1,2,3- and 1,2,4-TCB with concomitant release of chloride is shown in Figs. 1 and 2. The measured rate of 1,2,3- and 1,2,4-TCB degradation was respectively 2.49 and 2.27 nmol d^{-1} mg^{-1} dry weight of biomass during the first 4 d of incubation for cultures spiked with approximately 6 mg L^{-1} in the liquid medium of each pollutant. After 7 d of incubation, the percent of degradation was 91.1% (1,2,3-TCB) and 79.6% (1,2,4-TCB).

Since the toxicity of CBs increases with the number of chlorine substituents [23], microbial dechlorination of CBs is of major interest. Interestingly, the measured ratio for the micromoles of 1,2,4-TCB and 1,2,3-TCB degraded and chloride released was respectively 1:3.0 and 1:2.6, that indicates a near-complete dechlorination of the TCBs degraded. Neither the disappearance of TCBs nor the production of chloride ions was observed in heat-killed controls and in uninoculated bottles.

T. versicolor was not able to degrade 1,3,5-TCB under the conditions tested. This result is in accordance with other evidences found in the literature that showed the resistance of 1,3,5-TCB to aerobic transformation [24]. To the best of our knowledge, there is only one published report regarding the aerobic biodegradation of 1,3,5-TCB by two bacteria isolated from the tropical African environment [25].

1,2,4-TCB was selected as a model compound for further experiments since it is one of the most used chlorobenzenes and it also represents a possible intermediate product in the hexa- and pentachlorobenzene degradation pathway [1]. Moreover, 1,2,4-TCB is included in the list of the 31 priority chemicals of the US. EPA due to its high persistence, bioaccumulative properties and toxicity [26].

A study of the effect of 1,2,4-TCB concentration on its degradability by *T. versicolor* was carried out for 9 d. As shown in Table 1, degradation of 1,2,4-TCB was concomitant with release of stoichiometric amounts of chloride for the range of concentrations tested (6.5–23 mg L^{-1} in the liquid medium). A decrease in percent of degradation of 1,2,4-TCB was observed with the increase in 1,2,4-TCB concentrations, although the net degradation of 1,2,4-TCB was still greater at the higher concentrations. These results are particularly noteworthy since bacterial systems showed lower tolerance to TCBs concentration in comparison to *T. versicolor*. Thus, Adrian et al. [16] never observed dechlorination with 1,2,3- and 1,2,4-TCB concentrations exceeding 30 μM (~6 mg L^{-1}) in an anaerobic bacteria consortium. Holliger et al. [14] also did not observed dechlorination in an anaerobic mixed culture with concentrations higher than 40 μM of 1,2,3-TCB (~8 mg L^{-1}). Therefore, this is a distinct advantage with the white-rot fungus *T. versicolor* in comparison to the bacterial systems for bioremediation of CB-contaminated environments.

3.2. Extracellular enzymes and cytochrome P450 inhibition

In an effort to determine the major enzymatic system involved in 1,2,4-TCB metabolism, extracellular enzyme levels were measured. Neither MnP nor LiP activities were detected, although a maximum laccase activity of 298 AU L^{-1} was detected in *T. versicolor* cultures the third day of incubation. Thus, we examined the role of this enzyme by the addition of purified laccase from *T. versicolor*, but no oxidation of 1,2,4-TCB was observed. The addition of different mediators (HOBT, VA, DMHAP and ABTS) to the lac-

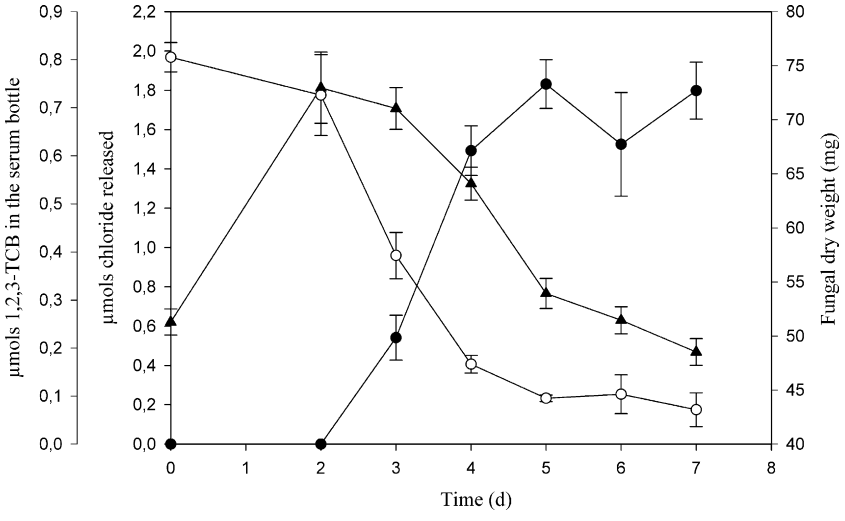

Fig. 1. Time course of 1,2,3-TCB degradation experiment by *T. versicolor*. Symbols mean micromoles of chloride released (●), micromoles of 1,2,3-TCB degraded (○) and biomass (fungal dry weight) (▲). The total amount of 1,2,3-TCB added initially was 0.805 ± 0.04 μmol for each serum bottle (approximately 6 mg L^{-1} in the liquid medium according the Henry's constant described in Section 2).

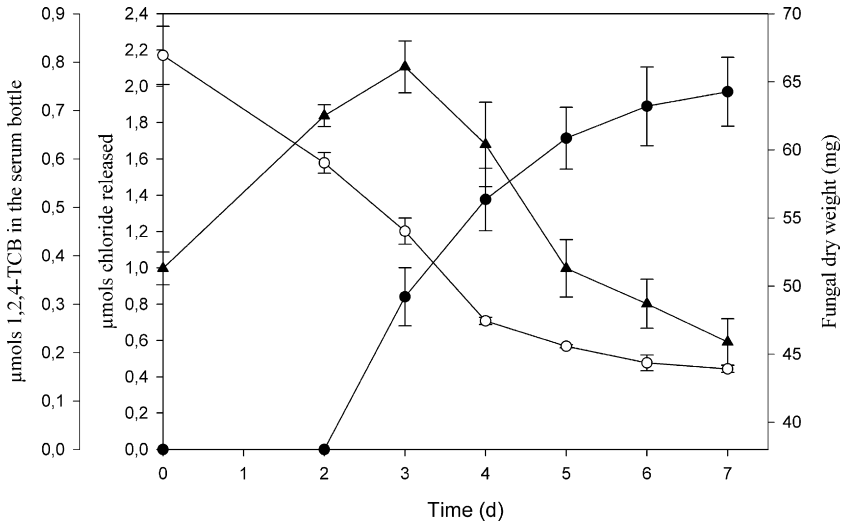

Fig. 2. Time course of 1,2,4-TCB degradation experiment by *T. versicolor*. Symbols mean micromoles of chloride released (●), micromoles of 1,2,4-TCB in the serum bottle (○) and biomass (fungal dry weight) (▲). The total amount of 1,2,4-TCB added initially was 0.814 ± 0.06 µmol for each serum bottle (approximately 6 mg L^{-1} in the liquid medium according the Henry's constant described in Section 2).

Table 1
Effect of different 1,2,4-TCB concentration on its degradation and release of chloride in cultures of *T. versicolor* after 9 d of incubation.

Initial 1,2,4,-TCB concentration (µmol)[a]	1,2,4-TCB degraded (µmol)	Chloride ion generated (µmol)	Correlation[b]	Percent degradation
0.905 ± 0.108	0.674 ± 0.095	2.25 ± 0.20	3.3	74
1.694 ± 0.042	1.035 ± 0.108	2.96 ± 0.16	2.9	61
2.261 ± 0.369	1.306 ± 0.028	4.29 ± 0.09	3.3	58
3.194 ± 0.089	1.565 ± 0.078	4.95 ± 0.23	3.2	49

[a] Total moles of 1,2,4-TCB added in the serum bottle. This amount is equivalent to 6.5, 12.2, 16.3 and 23.0 mg L^{-1} of 1,2,4-TCB in the liquid medium, respectively, according to the Henry's constants established in Section 2.4.

[b] This parameter establish the ratio between micromoles of chloride released and micromoles of 1,2,4-TCB degraded.

case system did not help to oxidize this pollutant. After that, it was assumed that the fungus may take up this substrate into the cell and that the degradation process may occur intracellularly. Therefore, a possible involvement of cytochrome P450 was examined using the cytochrome P450 inhibitors PB and ABT. As shown in Table 2 a strong inhibition of either dechlorination (>85%) and degradation (>75%) was clearly observed in those cultures containing PB and ABT after 9 d of incubation. No oxidation was observed in heat-killed controls. There were no statistical differences of fungal dry weights among treatments, which indicate that the addition of ABT and PB did not affect cell yields of *T. versicolor*. Thus, these results suggest that inhibition of 1,2,4-TCB degradation in cultures containing ABT and PB is not due to nonspecific inhibition of fungal growth and it appears that cytochrome P450 is responsible for the first oxidation step of 1,2,4-TCB.

These cytochrome P450 inhibitors have been shown to disrupt dibenzyl sulfoxide and diphenyl ether metabolism by *T. versicolor* [27,28], 4-nitrotoluene metabolism by *P. chrysosporium* [29] and 2,4,6-trinitrotoluene metabolism by *Bjerkandera adjusta* DSM 3375 [30]. In the last case, ABT did not affect the mineralization of [^{14}C]glucose, which is in agreement with the observation made here that growth proceeded well on defined medium in the presence of the inhibitors.

3.3. Identification of 1,2,4-TCB degradation metabolites

A study of the correlation between the 1,2,4-TCB degradation profile and chloride releases showed a slight decrease of 1,2,4-TCB the second day of incubation, but interestingly chlorides were not detected in the medium at this point (see Fig. 2). We hypothesized the possible transformation of 1,2,4-TCB to other chlorinated compounds at this time that would be further degraded with the consequent loss of chlorines through the incubation period. Thus, a screening for excreted metabolites of the culture supernatant the second and seventh day of incubation was performed.

Initially, ^{1}H NMR analysis of the control sample (heat-killed control), and samples after 2 and 7 d of incubation were carried out (see Supplementary data). In control and second day incubation samples, the 1,2,4-TCB structure was clearly observed, however none degradation compounds were detected. In both samples a COSY experiment was also performed in order to confirm the 1,2,4-TCB structure. In the case of the 7 d degradation sample, neither 1,2,4-TCB nor other degradation compounds were observed (see Supplementary data). Due to these results, the use of a more sensitive method was considered.

Thus, direct injection ESI-MS analyses of the former samples were performed. In order to compare the intensities of the mass

Table 2
Effect of the cytochrome P450 inhibitors ABT and piperonyl butoxide on 1,2,4-TCB degradation by *T. versicolor*.

Culture treatment	1,2,4-TCB degraded (µmol)	Chloride ion generated (µmol)	Fungal dry weight (mg)
ABT	0.155 ± 0.015	0.25 ± 0.08	35.5 ± 1.2
Piperonyl butoxide	0.114 ± 0.012	0.30 ± 0.15	33.5 ± 1.7
Inhibitor-free control	0.674 ± 0.095	2.25 ± 0.20	34.8 ± 0.2

Details regarding conditions of the experiments are found in Section 2.5.

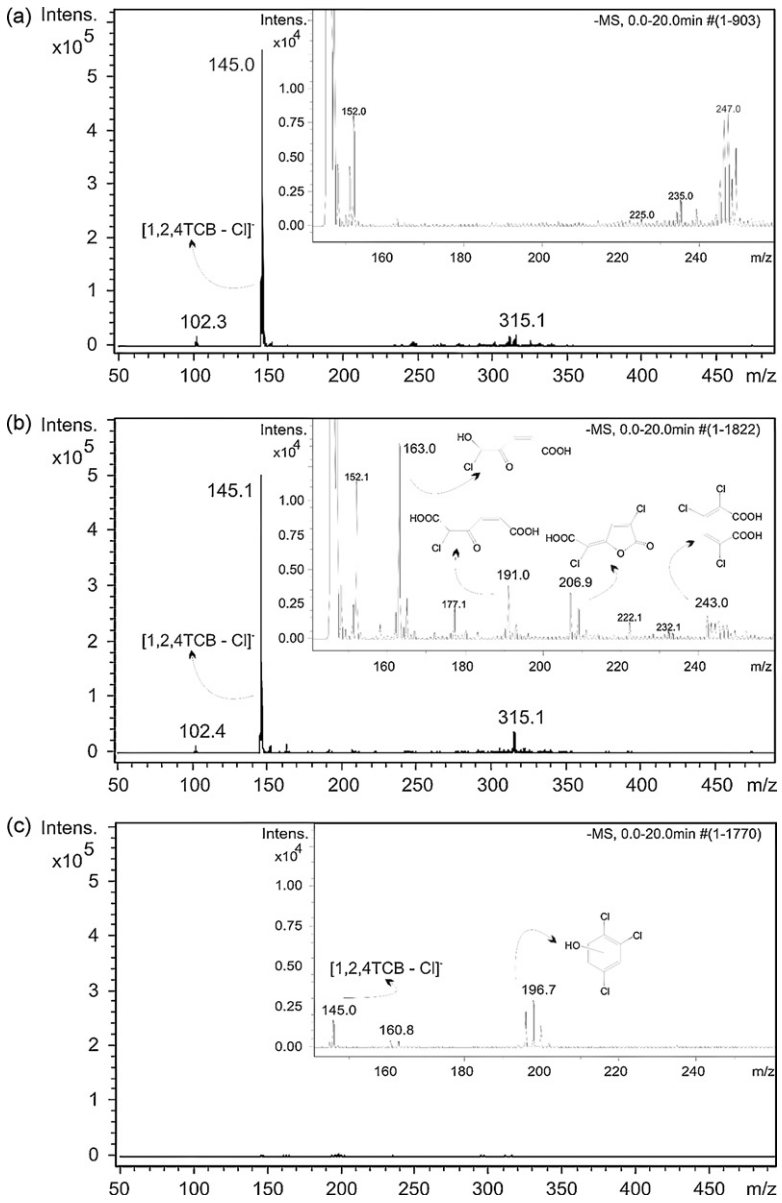

Fig. 3. Time course of 1,2,4-TCB degradation experiment by *T. versicolor* followed by ESI-MS. (a) Sample at initial time (it corresponds to the heat-killed control), (b) sample after 2 d of experiment and (c) after 7 d. All samples were prepared exactly under the same procedure and the spectra were acquired using the same method and conditions. Each spectrum is equally scaled and the enlargement of the m/z 140–460 region is shown.

spectroscopy signals, all three samples were prepared under exactly the same conditions and the analyses were acquired using the same method (see Section 2.7). Fig. 3 shows the mass spectra and the enlargement of the m/z 140–260 region for each sample. Every spectra show the same scale to facilitate the comparison. In control and 2 d degradation samples, 1,2,4-TCB in form of $[M-Cl]^-$ fragment ion (m/z = 145) was major detected, in accordance of what was previously observed by ^{1}H NMR. However, in the 2 d incubation sample, minor peaks not detected in the control sample were observed (see Fig. 3b), the most intense of those are the peaks at m/z = 243, 207, 191 and 163. Considering its m/z value and its isotopic pattern, they have been assigned to 2,3,5-trichloromucanate (m/z = 243), its corresponding carboxymethylenebutenolide (m/z = 207), 2- or 5-chloro-4-oxo-2-hexendioic acid (m/z = 191) and its degradation product 2- or 5-chloro-5-hydroxy-4-oxo-2-pentenoic acid (m/z = 163).

In the case of the 7 d incubation sample, two minor peaks were detected, one at m/z = 195, assigned to a trichlorophenol, due to its m/z value and its characteristic isotope pattern and a second peak at m/z = 145, assigned as in the other samples to 1,2,4-TCB $[M-Cl]^-$ fragment ion (see Fig. 3c).

Based on these results, a degradation pathway of 1,2,4-TCB by *T. versicolor* is proposed in Fig. 4, that is quite similar to that described previously by aerobic bacteria [7]. According this pathway, the initial oxidative attack of fungal cyt P450 system incorporate one atom of molecular oxygen into the aromatic double bonds to form an arene oxide that can either undergo enzymatic hydration by epoxide hydrolase to form the corresponding dihydrodiol (cis-3,4,6-trichloro-1,2-dihydroxycyclo-hexa-3,5-diene) or else rearrange non-enzymatically to form 2,4,5-trichlorophenol. This degradation mechanism is widely reported in the degradation of polycyclic aromatic hydrocarbons such as anthracene, phenanthrene, fluoranthene, chrysene, benz[a]anthracene among many others by the cyt P450 system of white-rot fungi (see a review in Cerniglia [31]). Our results showed that 2,4,5-trichlorophenol is a substrate of laccase (see Supplementary data) and the traces

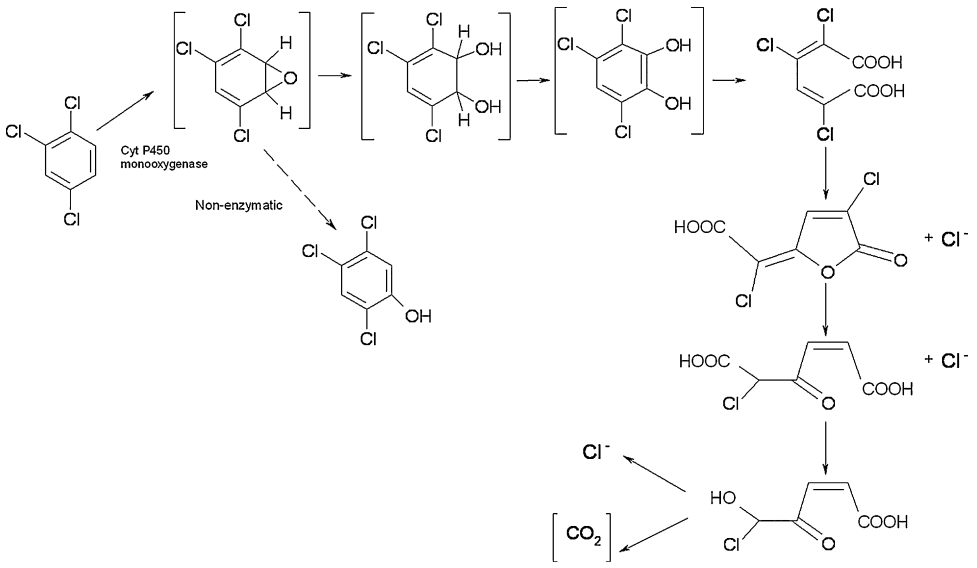

Fig. 4. Proposed degradation pathway of 1,2,4-TCB by *T. versicolor*. Chemicals set into square brackets were not identified in this work.

detected the seventh day of incubation could be due either to the deactivation of the laccase activity at the end of the incubation period or to an abiotic transformation from the dihydrodiol during extraction of the acidified medium, as was reported by Sander et al. [7]. The ability of laccase to dechlorinate other chlorophenols like 2,4,6-trichlorophenol has been also reported [32]. Regardless the origin of 2,4,5-trichlorophenol is noteworthy to say that it was found at trace levels and almost all 1,2,4-TCB degraded was completely dechlorinated after 9 of incubation, as is shown in Table 1.

It is postulated that further oxidation of cis-3,4,6-trichloro-1,2-dihydroxycyclo-hexa-3,5-diene resulted in the formation of 3,4,6-trichlorocatechol, that was subsequently converted to 2,3,5-trichloromuconate after an ortho cleavage pathway. From the trichloromuconate, under dechlorination, the corresponding carboxymethylenebutenolide was formed, which after hydrolysis and a dechlorination step, yielded 2- or 5-chloro-4-oxo-2-hexendioic acid. The decarboxylation of the latter plus an oxidation step led to 2- or 5-chloro-5-hydroxy-4-oxo-2-pentenoic acid or a structural isomer, that was completely dechlorinated in further steps according the chloride balance shown in Table 1.

4. Conclusions

The results presented here constitute the first demonstration of 1,2,3- and 1,2,4-TCB degradation under aerobic conditions by fungi in general, and more specifically by the white-rot fungus *T. versicolor*. Cytochrome P450 appears to be involved in the first step of 1,2,4-TCB oxidation by *T. versicolor*, according the experiments with cyt P450 inhibitors ABT and PB. Four intermediates of 1,2,4-TCB degradation were detected the second day of incubation by direct ESI-MS analysis: 2,3,5-trichloromuconate, a carboxymethylenebutenolide, 2- or 5-chloro-4-oxo-2-hexendioic acid and 2- or 5-chloro-5-hydroxy-4-oxo-2-pentenoic acid. The complete dechlorination of the 1,2,4-TCB degraded and the fact that the above metabolites were not detected in the supernatant after 7 d of incubation suggest complete mineralization of the substrate.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2008.12.076.

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