

1 Novel aerobic perchloroethylene degradation by the white-rot

2 fungus *Trametes versicolor*

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22

23 **Abstract**

24

25 Perchloroethylene (PCE) is one of the most important groundwater pollutants around the
26 world. It is a suspected carcinogen and is believed to be rather recalcitrant to microbial
27 degradation. We report here, for the first time, aerobic degradation of PCE by the white rot
28 fungus, *Trametes versicolor*, to less hazardous products. Aerobic degradation rate of PCE
29 was 2.04 to 2.75×10^{-4} $\mu\text{mol h}^{-1}$ mg dry weight of fungal biomass. Trichloroacetyl
30 chloride (TCAC) was identified as the main intermediate using [2-¹³C]PCE as the substrate.
31 Chloride release was stoichiometric with PCE degradation. Re-oxygenation of the cultures
32 resulted in increased PCE degradation as well as a corresponding increase in chloride
33 release. These results suggest that better degradation rates can be achieved by appropriate
34 optimization of culture conditions. Additionally, our studies using 1-aminobenzotriazole
35 (ABT), an inhibitor of cytochrome P-450, suggested that cytochrome P-450 system is
36 involved in PCE degradation by *T. versicolor*. These results are of particular interest
37 because both the involvement of cytochrome P-450 system in PCE degradation as well as
38 TCAC production from PCE has been reported to date only in mammalian systems, but not
39 in bacteria or fungi.

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45 **1. Introduction**

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47 Perchloroethylene (PCE), an effective degreasing solvent and fumigant, is among the most
48 frequently detected recalcitrant xenobiotic pollutant in soil and groundwater around the
49 world. In fact, PCE has been shown to be present most of the hazardous waste sites on the
50 U.S. Environmental Protection Agency National Priority List (1).

51 Although the reductive dechlorination of PCE has been achieved by bacterial
52 consortiums under anaerobic conditions some two decades ago (2- 5), description of axenic
53 cultures capable of degrading PCE have been more recent. These axenic cultures belong to
54 four different metabolic groups: halorespirers, acetogens, methanogens and facultative
55 anaerobes (6). Detailed studies on the mechanisms of reductive dehalogenation of PCE
56 have been initiated and several reductive dehalogenases involved in PCE degradation have
57 been purified (7-10). Co-metabolic dehalogenation, expected for acetogens and
58 methanogens, results in one step conversion of PCE to TCE and the release one chlorine
59 moiety. Most of the halorespirers, which are able to gain energy from the dehalogenation
60 reaction, and facultative anaerobes continues further conversion of PCE to less chlorinated
61 compounds. To date, only *Dehalococcoides ethenogenes* strain 195 has been known to
62 degrade PCE to the non-toxic compound ethene (11), while most other natural biotic and
63 abiotic processes degrade PCE to result in toxic products (such as cis-dichloroethylene) and
64 carcinogenic intermediates (such as vinyl chloride)(12-16).

65 For many years, PCE was thought to be non-biodegradable in the presence of
66 oxygen, but PCE degradation by *Pseudomonas stutzeri* OX1, involving a toluene-o-xylene

67 monooxygenase was reported recently (17, 18). The evidence for degradation was primarily
68 based on the quantification of chloride ions released into the medium, but the reaction
69 products were not identified. Also, Enzien et al (19) observed dehalogenation of PCE under
70 bulk aerobic conditions but they suggested that anaerobic microsites were the sites for
71 dehalogenation.

72 White rot fungi are able to degrade lignin present in woody plants using nonspecific
73 enzymes systems as exemplified by lignin peroxidases (LiP), manganese peroxidases
74 (MnP) and laccases (Lac). These enzymes use free radicals mechanisms to catalyze the
75 degradation of a wide variety of chloroaromatic pollutants (20-22). However, subsequent
76 studies have demonstrated that the mechanism of degradation of some pollutants are not
77 linked to the production of the peroxidase system and reported the involvement of alternate
78 oxygenases, particularly P-450 monooxygenases (23-25).

79 In this report, we demonstrate for the first time the ability of fungus to degrade PCE
80 under aerobic conditions using a degradation mechanism that has not been demonstrated in
81 microbes to date. We present here our results on PCE degradation rate, the reaction
82 products obtained, and a suggested mechanism for PCE degradation used by *T. versicolor*.
83 The feasibility for improvement in the degradation rate by reoxygenation of cultures of this
84 fungus has also been studied.

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87 **2. Materials and methods**

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89 **2.1. Fungal strains and chemicals.** *T. versicolor* (ATCC#42530) was maintained by
90 subculturing on 2% malt extract agar slants (pH 4.5) at room temperature. Subcultures were
91 routinely made every 30 days. PCE was obtained from Sigma-Aldrich Co. (St. Louis, MO)
92 and [2-¹³C]PCE (99%) was from Isotec (Miamisburg, OH).

93

94 **2.2. Media and cultures.** Defined medium contained 8 g/L glucose, 498 mg/L N as
95 ammonium tartrate, 10 and 100 mL/L of a micro and macronutrient solution, respectively
96 (26), and 1.168 g/L of 2,2-dimethylsuccinate buffer, unless otherwise indicated. pH was
97 adjusted to 4.5.

98 A mycelial suspension of *T. versicolor* was obtained by inoculation of four 1 cm²
99 area agar plugs from the growing zone of fungus on malt agar (2%) to a 500 ml Erlenmeyer
100 flask containing 150 ml of malt extract medium (2%) at pH 4.5. Flasks were incubated at
101 25°C on an orbital shaker (135 rpm, r=25 mm). After 4-5 days, the dense mycelial mass
102 was ground with a X10/20 homogenizer (Ystral GmbH, Dottingen, Germany). This blended
103 mycelial suspension was used as the inoculum. Pellets of *T. versicolor* were produced by
104 using 1 ml of the mycelial suspension to inoculate 250 ml of malt extract medium (2% malt
105 extract, pH 4.5) in a 1 litre Erlenmeyer flask. This was shaken (135 rpm, r=25 mm) at 25°C
106 for 5-6 days. Subsequently pellets formed by this process were washed with sterile
107 deionized water.

108

109 **2.3. PCE degradation experiments.** All the experiments were performed using 125-ml
110 serum bottles sealed with Teflon-coated grey butyl rubber stoppers (Wheaton, Millville,
111 N.J.) and aluminium crimps (Baxter Scientific Products, McGaw Park, Ill). Each bottle was
112 inoculated with 2 g of wet pellet of *T. versicolor* (equivalent to 5.0 g/l dry weight). 10-ml
113 of liquid medium was added to each inoculated bottle and subsequently was oxygenated for
114 1 min (30 KPa) and sealed immediately. Then, 20 μ L of a solution of PCE in ethanol was
115 added by means of a pressure-lok gas-tight syringe (VICI Precision Sampling, Baton
116 Rouge, LA) through the stoppers to give 5 mg/L PCE in the liquid media. The bottles were
117 shaken vigorously for 30 min in an inverted position (to minimize gas leakage) and
118 subsequently were incubated at 25°C on an orbital shaker (135 rpm, r=25 mm), also in an
119 inverted position. In those cases where reoxygenation took place, 5 ml of pure oxygen was
120 added by means of a pressure-lok gas-tight syringe through the stoppers.

121 Each experiment included uninoculated and heat-killed controls. Heat-killed
122 controls consisted of autoclaved cultures that had been pre-grown for 7 days under
123 conditions identical to those of the experimental cultures. Percent degradation at a specified
124 interval was calculated by comparing concentration in the uninoculated blanks with those in
125 the experimental bottles. All degradation values were corrected for the sorption values
126 determined using the heat-killed controls. PCE concentration values were also corrected
127 considering the water volume added with pellets. Each bottle was sacrificed at each time
128 point for analysis.

129

130 **2.4. Experiments with cytochrome P-450 inhibitor.** For those microcosms that were
131 tested with the cytochrome P-450 inhibitor 1-aminobenzotriazole (ABT), a final

132 concentration of 1 mM ABT was present in 10 ml of defined medium with 2 g of *T.*
133 *versicolor* pellets, as described above. The bottles were incubated at 25°C on an orbital
134 shaker (135 rpm) for 7 days. Heat-killed and inhibitor-free controls were included in
135 triplicate in this experiment.

136

137 **2.5. PCE analysis.** The concentration of PCE was determined by static headspace gas
138 chromatography. All samples were equilibrated at 25°C before analysis. A 1 ml liquid
139 sample from each experimental bottle was transferred to 4 ml sodium azide solution (1%)
140 in a 10 ml vial and sealed immediately with a teflon coated stopper. The vial was placed in
141 a headspace sampler Agilent 7964 (Agilent Technologies, Palo Alto, CA) and was heated to
142 85°C for 50 min. Subsequently, a 1-mL headspace sample was injected automatically into a
143 gas chromatograph (Agilent 6890N) equipped with a column Agilent HP-5 (30 × 0.32 ×
144 0.25) and a flame ionization detector.

145 The GC operating conditions were as follows: column temperature, 40°C (2 min),
146 slope 4°C/min, 50°C (1 min), slope 10°C/min, final temperature: 160°C; injector
147 temperature, 125°C; flame ionization detector temperature, 260°C; and carrier gas He at 7
148 psi pressure. Data was acquired and quantified by Millennium 32 software (Waters,
149 Milford, MA).

150 Total amount of PCE in the experimental bottles and its concentration in liquid
151 media were determined by comparing peak areas with those of external standards and by
152 using Henry's law constant reported previously (27) and verified in our laboratory.

153

154 **2.6. GC-MS analysis.** GC-MS measurements were performed injecting a 1-mL headspace
155 sample automatically from a headspace sampler Agilent 7964 (Agilent Technologies, Palo
156 Alto, CA) using same conditions as those described above, to an Agilent 6890 (Agilent
157 Technologies, Palo Alto, CA) gas chromatograph coupled with an Agilent 5973 mass
158 spectrometer (Agilent Technologies, Palo Alto, CA). The samples were injected on
159 capillary column Agilent HP-5MS (30 × 0.25 × 0.25), and helium was used as the carrier
160 gas. The temperature program was 40°C (2 min), slope 4°C/min, 50°C (1 min), slope
161 10°C/min, final temperature: 200°C and injector temperature, 125°C. The following MS
162 conditions were used: ionization mode, EI⁺; ionization energy, 70 eV; mass range, m/z 35
163 to 220.

164 Intermediates were identified using the Wiley 275 Mass Spectral Library (John
165 Wiley & Sons, New York, NY; purchased from Hewlett Packard, Palo Alto, CA) and by
166 comparison of the mass spectra with those of a commercially available pure compound.

167

168 **2.7. NMR analysis.** The identification of PCE degradation intermediates was done using
169 [2-¹³C]PCE in experiments similar to those described above followed by nuclear magnetic
170 resonance (NMR) analysis. NMR spectra were recorded in a BRUKER AV500
171 spectrometer equipped with a high-sensitivity cryogenically cooled TCI probe and
172 operating at 100.62 MHz for ¹³C.

173 The sample used was prepared by adding 50µl of D₂O (as a source of deuterium to
174 lock the sample into the magnet) in a 500µl of a 31-day old liquid sample. ¹H-decoupled
175 ¹³C spectra were recorded using the inverse-gated method with 5 seconds of pre-scan delay.
176 Data were processed using an exponential window function (line broadening of 2 Hz) prior

177 to Fourier Transformation. The resulting NMR spectra were compared with those of non-
178 labelled standards to confirm the presence or absence of possible target compounds.

179

180 **2.8. Other analyses.** Mycelial dry weights were determined by vacuum filtering the
181 cultures with preweighed glass filters (47-mm-diameter). The filters containing the
182 mycelial mass were placed in glass dishes and dried at 100°C to constant weight.

183 The concentration of chloride ions released during PCE degradation was measured
184 by an ionic chromatograph Dionex ICS-1000 equipped with a conductivity detector
185 (Dionex, Wommelgem, Belgium), using a 4-mm anionic exchanger column, IonPack AS9-
186 HC (also from Dionex). The volume of injection was 25 µL and the mobile phase was
187 9 mmol/L sodium carbonate solution with a flow rate of 1 ml/min.

188 Laccase activity was measured using a modified version (28) of the method for the
189 determination of manganese peroxidase (29), where 2,6-dimethoxyphenol (DMP) was
190 oxidized by laccase in the absence of a cofactor. Conversely, oxidation by manganese
191 peroxidase (MnP) requires the presence of H₂O₂ and catalytically active Mn²⁺. One activity
192 unit (AU) was defined as the number of micromoles of DMP oxidized per minute. The
193 DMP extinction coefficient was 10000 M⁻¹ cm⁻¹.

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195

196 **3. Results**

197

198 **3.1. Degradation of PCE by *T. versicolor* and identification of the primary**
199 **intermediate.**

200 Results presented in Figure 1 and 2 show that substantial PCE degradation observed in
201 experimental flasks was accompanied by the production of a product which was
202 subsequently identified as chloroform (see below). The measured rate of PCE degradation
203 was between 2.04 and $2.75 \times 10^{-4} \mu\text{mol h}^{-1}\text{mg}^{-1}$ dry weight of biomass during the first three
204 days of incubation. Neither the disappearance of PCE nor the production of any
205 intermediate was observed in heat-killed controls and uninoculated bottles.

206 Laccase activity is strongly inhibited by the addition of PCE, obtaining a maximum
207 enzyme activity of $45.5 \pm 4.3 \text{ AU/l}$ (3rd day of incubation) and 16.2 ± 7.1 the remaining
208 period. In control cultures under identical growth condition but without PCE the maximum
209 enzyme activity was $226.2 \pm 13.5 \text{ AU/l}$.

210 The intermediate detected during PCE degradation was identified as chloroform
211 based on GC-MS analysis and using the Wiley 275 Mass Spectral Library. The mass
212 spectrum of chloroform is characterized by the peaks at m/z 47, 83 and 118 (Figure 3A).
213 This mass spectrum coincided fully with that obtained during PCE degradation experiment
214 (Figure 3B). In stable isotopic-labelling degradation experiments with $[2\text{-}^{13}\text{C}]\text{PCE}$,
215 chloroform carried the ^{13}C label. Their mass spectra showed a shift of 1 atomic mass unit
216 compared to the chloroform obtained from non labeled PCE (47→48, 83→84, 118→119)
217 as shown in Figure 3C.

218

219 **3.2. Identification of intermediate by nuclear magnetic resonance (NMR).** Appearance
220 of chloroform was not reported in previous PCE biodegradation reports. Our working
221 hypothesis was that chloroform was formed by an abiotic process possibly from another
222 primary intermediate during static headspace gas chromatography analysis. Therefore,
223 cultures spiked with 5 mg/L of [2-¹³C]PCE were analyzed by ¹³C{¹H} NMR. The presence
224 of [¹³C]trichloroacetic acid ([¹³C]TCA) was confirmed by the presence of an AX spin
225 system consisting of two doublet resonances at chemical shifts of 167.1 and 95.4 ppm with
226 a J(CC) coupling value of 61Hz (Figure 4B). These resonances agreed with the NMR
227 spectrum obtained from a sample of commercially available, non-labeled TCA. In addition,
228 the doublet splitting of these resonances confirms that TCA arises from the initial [2-
229 ¹³C]PCE. On the other hand, while NMR signals from [2-¹³C]PCE, and non-labeled α- and
230 β-glucose and ethanol were clearly visible in heat-killed controls, TCA resonances were not
231 observed in these cultures (Figure 4A).

232

233 **3.3. Inhibition study with ABT.** The addition of 1-aminobenzotriazole (ABT), a known
234 inhibitor of cytochrome P-450 system (30), to cultures containing 5 mg/L of PCE resulted
235 in total inhibition of PCE degradation whereas in inhibitor-free controls, PCE degradation
236 and chloride release was seen (Table 1). No PCE degradation was observed in heat-killed
237 controls. In parallel experiments, ABT did not affect cell yields of *T. versicolor* (data not
238 shown)

239

240 **3.4. Effect of oxygenation on PCE degradation and chloride release.** Since *T. versicolor*
241 is an aerobic organism, we hypothesized that oxygen depletion may be a significant
242 limitation on PCE degradation in the closed culture vessels employed in this study,
243 necessitated by the fact that PCE is highly volatile. The observed plateau in PCE
244 degradation observed after 6 days of incubation is consistent with this idea. Therefore, one
245 set of parallel cultures were re-oxygenated after 4 days of incubation. These re-oxygenated
246 cultures showed a slight increase in PCE degradation as well as in the amount of chloride
247 ions released. (Table 2).

248

249

250 **4. Discussion**

251

252 Involvement of cytochrome P-450 in PCE degradation has so far been reported in
253 mammalian systems only. No anaerobic or aerobic bacteria were shown to catalyze P-450
254 mediated degradation of PCE. The results presented here constitute the first demonstration
255 of PCE degradation under aerobic conditions by fungi in general, and more specifically the
256 white-rot fungus *T. versicolor*. That PCE degradation observed is biological is supported by
257 the following observations: (1) increase in PCE degradation occurs during the first three
258 days, which coincides with the product formation (see Figure 1 and 2); (2) the
259 stoichiometry between μmol of chloride released and μmol of PCE degraded was
260 comparable to the theoretical ratio of 1:1 (see Table 2); (3) dechlorination of PCE does not
261 occur in heat-killed controls and in uninoculated bottles; and (4) Oxidative degradation of
262 PCE and concomitant chloride release and TCAC production was inhibited in the presence
263 of 1-aminobenzotriazole (ABT), a known cytochrome P-450 inhibitor.

264 The results on PCE disappearance, concomitant chloride release, and $[2\text{-}^{13}\text{C}]\text{PCE}$
265 experiments show that PCE is degraded by *T.versicolor* to trichloroacetyl chloride (TCAC),
266 which is rapidly hydrolyzed in water (abiotically) to TCA. The later compound undergoes
267 spontaneous intramolecular rearrangement at elevated temperature (85°C), which occurs
268 when the culture sample is heated during gas chromatography, and forms chloroform. This
269 abiotic formation of chloroform is some what analogous to the formation of
270 trichloroacetaldehyde (chloralhydrate) from trichloroethylene (31). Formation of

271 chloroform from TCAC in this study (Figure 2) was independently corroborated by
272 injecting pure TCAC with deionized water using static head space gas chromatography.

273 It is note worthy that vinyl chloride and dichloroethene, frequently seen products of
274 reductive dehalogenation by bacteria under anaerobic conditions, were not observed as
275 products of PCE degradation by *T. versicolor*. In mammalian systems, cytochrome P-450
276 mediated oxidation of PCE results in the formation of TCAC via epoxy-PCE, which reacts
277 subsequently with amino groups in macromolecules or with water to give trichloroacetic
278 acid (TCA) (32, 33). Thus, PCE degradation by *T. versicolor* appears to be analogous to the
279 mammalian systems and is quite different from PCE degradation aerobic and anaerobic
280 bacteria described to date. Furthermore, unlike PCE and its products of vinyl chloride and
281 dichloroethene seen in bacterial systems, TCA produced from PCE by cultures of *T.*
282 *versicolor* is not considered to be a suspected carcinogen, is far less toxic, and is readily
283 degraded by other organisms in the environment. This is a distinct advantage with the
284 white-rot fungus *T. versicolor* in comparison to many of the bacterial systems for
285 degradation of PCE.

286 Enzymes of the P-450 super family are found in a wide range of prokaryotic and
287 eukaryotic organisms and have been well characterized, regarding their function,
288 regulation, and expression (34, 35). In mammalian systems, PCE is known to be
289 metabolized by both cytochrome P-450- and glutathione-dependent biotransformation
290 pathways, leading to the generation of reactive metabolites which may covalently bind to
291 cellular macromolecules (32, 33, 36, 37). In contrast to this, relatively little is known about
292 the basic biochemistry of fungal P-450 systems, in spite of the fact that P-450 has been
293 known for several years to play a key role in the biotransformation of various

294 environmental pollutants (24, 25, 38, 39) by white-rot fungi. Our experiments with
295 cytochrome P-450 inhibitor, ABT suggest that *T. versicolor* degrades PCE by the pathway
296 presented in Figure 5. Our data supporting the formation of TCAC and TCA as successive
297 degradation products of PCE is supported by the earlier precedent of PCE transformation to
298 TCAC in mammalian systems. Recent reports on the identification and characterization for
299 the first time of P-450 encoding genes in *T. versicolor* (40), and the reported involvement
300 of P-450 in this organism in metabolizing recalcitrant dibenzothiophene derivatives (41), is
301 also consistent with the results of this study suggesting the involvement of P-450 in the
302 PCE degradation pathway shown in Figure 5.

303 Three major families of lignin-modifying enzymes, lignin peroxidases (LiP),
304 manganese peroxidases (MnP), and laccases have been recognized (42). These enzymes are
305 relatively non-specific with respect to substrate and one or more of these enzymes are
306 known to catalyze the degradation of a wide variety of chroaromatic pollutants (42). No
307 LiP or MnP activity was detected in the extracellular culture fluid in any of the *T. versicolor*
308 cultures in this study. Laccase activity was found but the level of its activity in PCE
309 cultures was much less than that observed in uninoculated control cultures without PCE.
310 Addition of laccase in vitro to TCAC containing reaction mixtures, in the presence or
311 absence of mediators such as 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)
312 diammonium salt (ABTS), hydrate 1-hydroxy-benzotriazol (HOBT), voluric acid (VA) and
313 3,5-dimethoxy-4-hydroxyacetophenol (DMHAP) [data not shown], did not result in TCAC
314 degradation. Furthermore, adding TCAC (5 mg/L) to growing cultures of *T. versicolor* did
315 not result in its degradation. These results indicate that TCAC (or TCA) are not degraded

316 further by *T. versicolor* cultures; however, both TCA and TCAC are known to be
317 efficiently degradable by pure and mixed cultures of other microbes (43-47).

318 Compared with dehalorespirers, co-metabolic PCE dechlorination processes
319 proceed at a much lower rate. The rates of PCE dechlorination (to TCE) by
320 *Methanosarcina sp.* and *Acetobacterium woodii* were 3.5×10^{-5} and 3.6×10^{-3} $\mu\text{mol h}^{-1}$ mg
321 protein⁻¹, respectively (48). In comparison to this, PCE dechlorination rates coupled to
322 dehalorespiration in *Dehalospirillum multivorans* and *Dehalococcoides ethenogenes* strain
323 195 were 3 (49) and 4.14 $\mu\text{mol h}^{-1}$ mg protein⁻¹ (11), respectively. Considering that 10% of
324 the mycelial dry weight of filamentous fungi is protein (50), the PCE dechlorination rate (to
325 TCAC) by *T. versicolor* was calculated to be 2.04 to 2.75×10^{-3} $\mu\text{mol h}^{-1}$ mg dry weight of
326 biomass⁻¹, which is closer to that of bacteria which degrade PCE co-metabolically but lower
327 than that of dehalorespirers.

328 In this study, we observed that much of the growth in *T. versicolor* cultures occurs
329 in the first three days and much of the oxygen gets depleted in these cultures around this
330 time (data not shown) resulting in lower PCE degradation during the rest of the incubation
331 period. In an earlier study, similar cultures grown in serum bottles with the white-rot
332 fungus *P. chrysosporium* showed 95.4% consumption of available oxygen in 5 day-old
333 cultures (51). This indicated the importance of re-oxygenation for potential improvement of
334 the PCE degradation by *T. versicolor*. Consistent with this, cultures that were re-
335 oxygenated after four days of cultivation showed higher level of degradation and chloride
336 release than control cultures (Table 2) but not as much as one would have expected
337 suggesting that there may also be other factor(s) that might be contributing to the limitation
338 of PCE degradation in older cultures.

339 The results of this study open-up an interesting new area for detailed studies on the
340 physiology, biochemistry, and molecular biology of aerobic degradation of PCE by white-
341 rot fungi, a group that has been and is being studied intensively for their versatility in
342 degrading a variety of chlorinated environmental pollutants (42). Such studies are
343 particularly important since most of the work to date primarily focused on bacterial PCE
344 degradation by reductive dehalogenation. Given the fact that *T. versicolor* is an ubiquitous
345 fungus worldwide, it would be an interesting model for further studies and it could
346 potentially be an important organism in the future for bioremediation of PCE-contaminated
347 environments.

TABLE 1. Effect of the cytochrome P-450 inhibitor ABT on PCE degradation by *T. versicolor*^a

Culture Treatment	PCE in the serum bottle (μmol)	Cl⁻ generated (μmol)
ABT-free cultures	1.95 ± 0.05	0.8 ± 0.3
Cultures containing 1mM ABT	3.01 ± 0.21	-
Heat-killed controls	2.62 ± 0,23	-

^aDetails regarding conditions of the experiments are found in Materials and Methods. Values represent means ± standard deviations for triplicates.

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TABLE 2. Effect of re-oxygenation on PCE degradation and the release of chloride ions in cultures of *T. versicolor* ^a.

Treatment	PCE degraded (μmol)	Cl⁻ generated (μmol)
Re-oxygenated cultures	1.4 \pm 0.08	1.4 \pm 0.6
Non-re-oxygenated cultures	1.2 \pm 0.03	1.1 \pm 0.2

^aCulture conditions were as described in the legend to Figure 1. Degradation was measured at 2-day intervals and one serum bottle was sacrificed at each time point for analysis. On the 4th day of cultivation, 5 ml of pure oxygen was added in the re-oxygenated bottles. Values presented are means \pm standard deviations for three samples analyzed after 8-days of incubation when %PCE degradation for re-oxygenated bottles has reached a plateau. All values are corrected for sorption values obtained with parallel heat-killed controls.

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365 **FIGURE LEGENDS**

366

367 **FIGURE 1.** Degradation of PCE by *T. versicolor*^a.

368

369 **FIGURE 2.** Appearance of an intermediate identified primary as chloroform during PCE
370 degradation by *T. versicolor*^b.

371

372 **FIGURE 3.** Mass spectra of chloroform. (A) Mass spectrum of commercially available
373 chloroform. (B-C) Mass spectra of chloroform produced in degradation experiments with
374 non-labelled PCE (B) and [2-¹³C]PCE (C).

375

376 **FIGURE 4.** NMR spectra for identification of PCE-degradation reaction products. Spectra
377 for heat-killed controls (A) and cultures of *T. versicolor* (B) spiked with 5 mg/L of [2-
378 ¹³C]PCE.

379

380 **FIGURE 5.** Suggested pathway for PCE degradation to Trichloroacetic acid (TCA) by the
381 white rot fungus *T. versicolor*.

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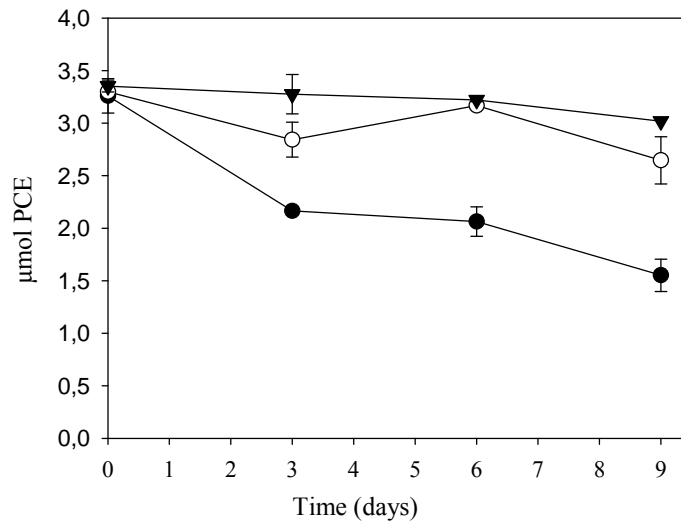
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FIGURE 1

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389

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391 ^a 10-ml of liquid media and 2 g of wet *T. versicolor* pellet were added in each serum bottle.

392 PCE concentration was 5 mg/L in the liquid media. Cultures were incubated in shaken

393 conditions (135 rpm) at 25°C in serum bottles sealed with Teflon-coated stoppers. Values

394 plotted are means \pm standard deviations for triplicate cultures. Individual bottles were

395 sacrificed at each sampling time. Symbols mean μ mol of chloroform (\square), and μ mol of

396 PCE in uninoculated bottles (\blacktriangledown), in heat-killed controls (\circ), and experimental bottles (\bullet).

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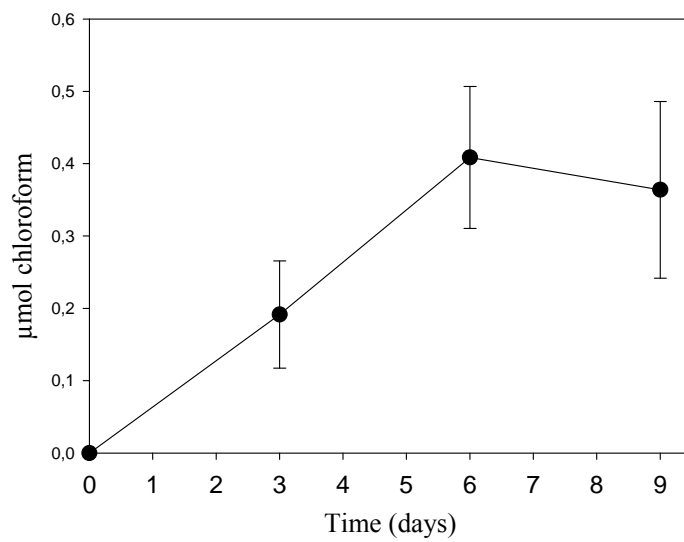
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FIGURE 2



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^a Culture conditions are the same than those described in Figure 1.

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FIGURE 3

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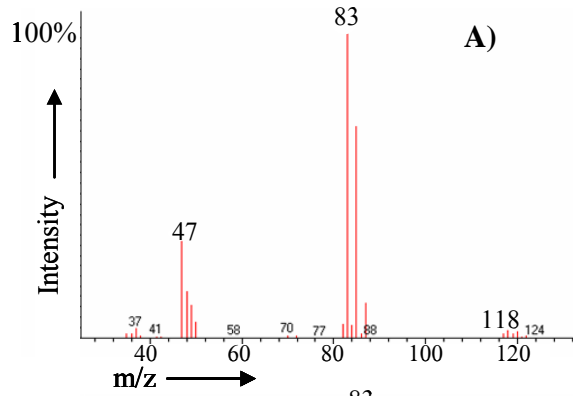
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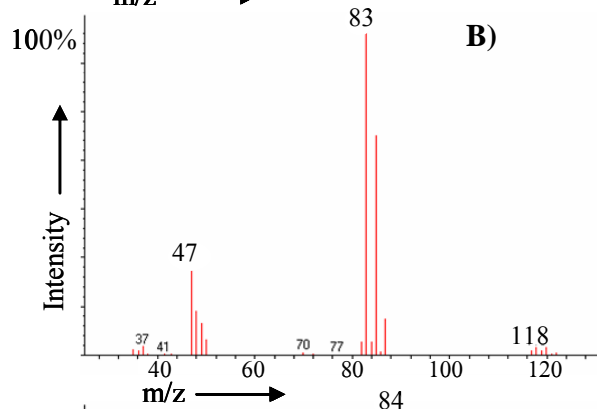
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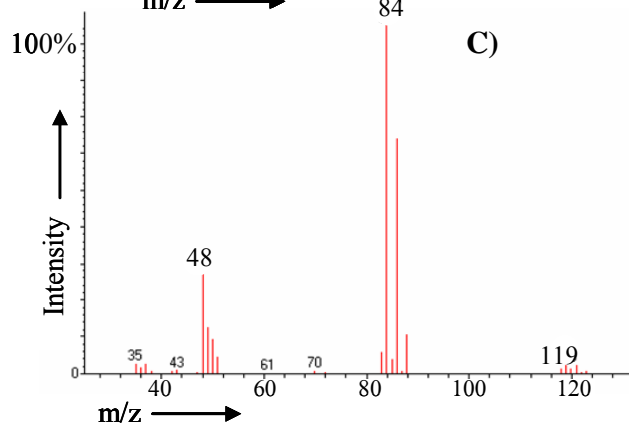
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425 ^a Culture conditions were as described in the legend for Figure 1. Samples were analyzed

426 after 7 days of incubation.

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FIGURE 4

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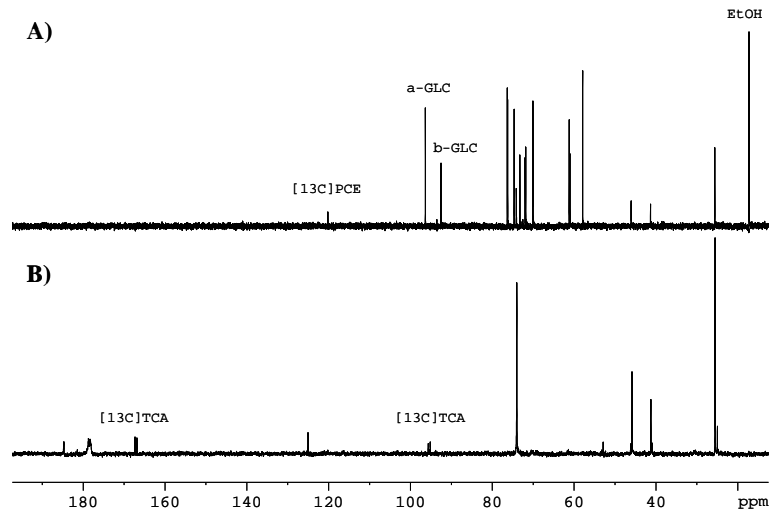
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440 ^aCulture conditions were as described in the legend for Figure 1, but [2-¹³C]PCE was used
441 in this case. Samples were analyzed after 7 days of cultivation.

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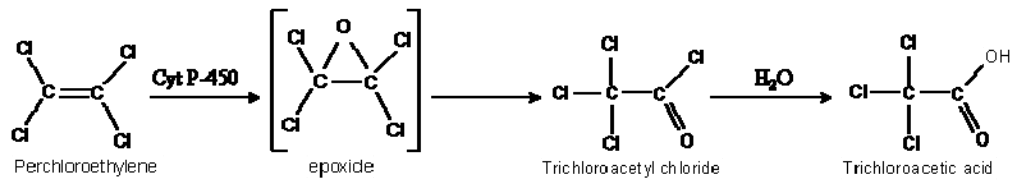
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FIGURE 5

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