

Degradation of tribromophenol by wood-rot fungi and hamilton system

Maríel Monrroy

Centro de Biotecnología
Laboratorio de Recursos Renovables
Facultad de Ciencias Forestales
Universidad de Concepción
Casilla 160-C
Concepción, Chile
Tel: 56 41 204601
Fax: 56 41 247517
E-mail: mmonrroy@udec.cl

Juanita Freer

Centro de Biotecnología
Laboratorio de Recursos Renovables
Facultad de Ciencias Químicas
Universidad de Concepción
Casilla 160-C
Concepción, Chile
Tel: 56 41 204601
Fax: 56 41 247517
E-mail: jfreer@udec.cl

Jaime Baeza

Centro de Biotecnología
Laboratorio de Recursos Renovables
Facultad de Ciencias Químicas
Universidad de Concepción
Casilla 160-C
Concepción, Chile
Tel: 56 41 204601
Fax: 56 41 247517
E-mail: jbaeza@udec.cl

Jaime Rodríguez*

Centro de Biotecnología
Laboratorio de Recursos Renovables
Facultad de Ciencias Forestales
Universidad de Concepción
Casilla 160-C
Concepción, Chile
Tel: 56 41 204601
Fax: 56 41 247517
E-mail: jrodrig@udec.cl

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Abbreviations: DHB: dihidroxybencenes
EPA: Environmental Protection Agency
PCPNa: sodium pentachlorophenate
TBP: 2,4,6-tribromophenol
TBPNa: sodium tribromophenate

Biological, chemical and combined treatments were used to degrade TBP. The biological treatment consisted in the use of *Laetiporus sulphureus*, *Gloeophyllum trabeum* and *Ganoderma australe*, which respectively

achieved 48%, 74% and 80% degradation, and 40%, 70% and 77% of organic bromine removal (AOX) on TBP water solutions (60 mg L⁻¹) after 15 days of bio treatment. The biological treatment with *G. australe* on

* Corresponding author

TBP-contaminated sawdust (10 mg kg⁻¹) led to 23% degradation. The chemical treatment consisted in the Hamilton system (Fenton reaction assisted by 1,2-dihydroxybenzene); observing with this treatment, 95% degradation, 50% mineralization (TOC), and 48% reduction of chemical oxygen demand (COD). The combined treatment of both (chemical-biological) treatments produced degradations of 100%, and reduction of toxicity of 34% and 30%, with *L. sulphureus* and *G. australe*, respectively.

In 1998, TBP, a compound of acute toxicity, was incorporated by the Environmental Protection Agency (EPA, USA) to the list of hazardous wastes (EPA, 1998). This compound is used as a large-scale biocide, and especially as a wood preservative. In Chile, TBPNa has been used in recent years, as substitute to PCPNa to prevent the wood stained chromogenous fungi (CONAMA, 2000). One result of its application is that TBP is frequently found in soils, sawdust and waters, where it is toxic and dangerous for human health and the environment. One technology that has been successfully used in the decontamination of soils and waters containing recalcitrant compounds is the application of wood-degrading fungi (Yadav and Reddy, 1993; Eggen, 1999; Schützendübel et al. 1999; Shollosser et al. 2000; Newcombe et al. 2002). In degradation processes, in addition to enzymatic systems, these fungi have advanced oxidation systems, such as the Fenton reaction (Shollosser et al. 2000; Jensen et al. 2001), which are assisted by low molecular mass compounds, such as DHB, that have the capacity to reduce and link metals (Shimada et al. 1997; Paszczynski et al. 1999; Qian et al. 2002; Wang and Gao, 2003).

This study evaluated the TBP degradation by a biological treatment (using *Ganoderma australe*, *Gloeophyllum trabeum* y *Laetiporus sulphureus*) and a chemical treatment with 1,2-DHB/Fe³⁺/H₂O₂ simulating the advanced oxidation system of fungi. The combined chemical-biological treatment was also tested.

MATERIALS AND METHODS

Biological treatment

Fungal strains *G. trabeum* ATCC 11539, *L. sulphureus* ATCC 52600 and *G. australe* IJFM, CIB-CSIC (Madrid, Spain) were pre-grown in Petri dishes with dextrose potato agar medium (3.9%) at 25°C for 10 days. Subsequently, 3 discs (10 mm of diameter) of mycelia were removed and added to a 125-mL Erlenmeyer flask containing 25 mL liquid culture (buffered with 20 mM 2,2-dimethylsuccinic acid sodium salt at pH 4.5 and incubated at 25 rpm at 25°C for 3 days). Then, TBP solutions (5% ethanol) were added to liquid culture to produce a final concentration of 60 mg L⁻¹. Flasks were incubated at 25 rpm for 20 days, quantifying every 4 days. The liquid culture was filtered with glass wool and 0.22 µm nitrocellulose filter. The filtered was

analyzed by high-performance liquid chromatographic (HPLC) and adsorbable organic halides (AOX).

For TBP degradation in sawdust, 8 g sawdust of *Eucalyptus globulus*, previously hydrated were mixed with 1 mL TBP solution for 10 µg TBP/sawdust g (dry weight) concentration, pH was adjusted to 5, incubated for 1 day and then inoculated with 18 mg of *G. australe* (dry weight) and incubated to 25°C for 20 days. TBP concentration was determined every 5 days by gas chromatography with electron detector (GC-ECD).

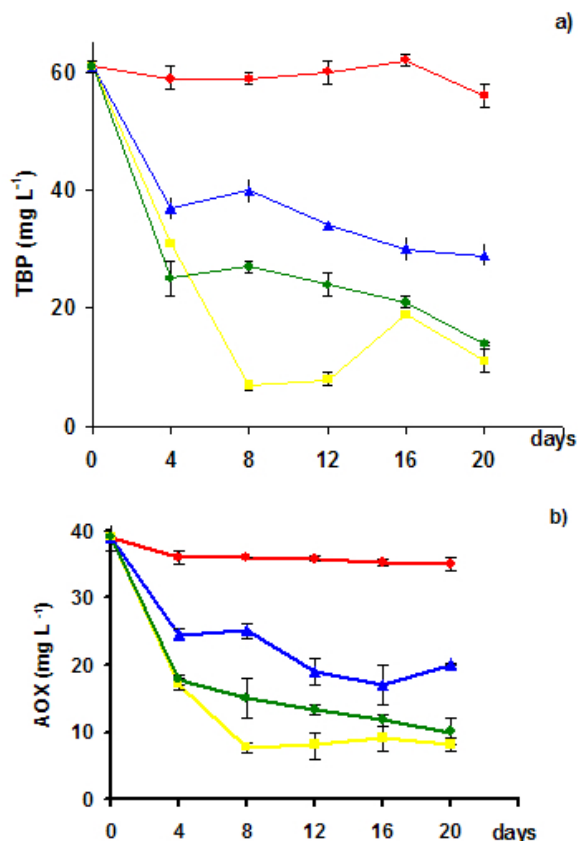


Figure 1. Concentration (mg L⁻¹) in liquid culture for *G. trabeum* (●), *L. sulphureus* (▲) and *G. australe* (■). Control (●): (a) TBP and (b) AOX. (Value represents means ± S.D. for triplicate cultures).

Chemical treatment

To 10 ml TBP (60 mg L⁻¹), the following were added in the order indicated 25 µM 1.2 DHB, 38 µM FeCl₃.H₂O, 980 µM H₂O₂ (Hamilton system), pH adjusted to 3, and kept at 25°C in the dark. TBP degradation was evaluated every 1 hr for 10 hrs by HPLC and total organic carbon (TOC). Toxicity (MICROTOX®) and chemical oxygen demand (COD) were measured at the end of the reaction. Treatment optimal conditions for Hamilton system were previously determined by multivariate analysis in other study in our laboratory (Rojo et al. 2004).

Chemical-biological treatment

Mycelium of fungi previously grown in a liquid medium for three days, as described earlier, was washed with abundant sterilized water, and then added to chemically treated samples (during 10 days). Later, inoculated samples were incubated at 25 rpm, 30°C for 5 days. The samples were analyzed by HPLC and MICROTOX ®.

Analytical procedures

TBF degradation was monitored by HPLC and GC-ECD. AOX was determined in Euroglas ESC 1000. Mineralization was monitored using a Shimadzu TOC-5050 system and COD by spectrophotometric UV-VIS. For acute toxicity determination luminescent bacteria were used, specifically the strain *Vibrio fischeri* and MICROTOX ® system.

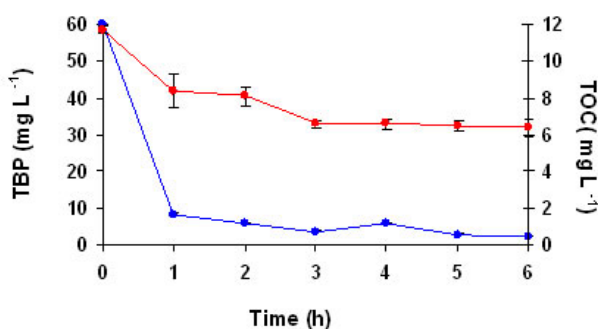


Figure 2. TBP Degradation (●) and mineralization (●) by Hamilton system. (Value represents means ± S.D. for triplicate culture).

RESULTS AND DISCUSSION

Biological treatment

Degradation in liquid culture showed that in the 4 first days of fungi treatment, TBF was degraded almost in a 50%, being significantly greater the degradation with *G. trabeum* in first day. For the following days, *G. australe* presented a greater degradation percentage. After 20 days of treatment with *L. sulphureus*, *G. trabeum* and *G. australe*, the samples presented 48%, 74% and 80% degradation, respectively (Figure 1). *G. australe* presented a significantly higher capacity degrading the compound compared to the other fungi. In all treated samples, the AOX diminished considerably (Figure 2), around 70%, 77% and 40% of removal of organic bromine was reached by *G. trabeum*, *G. australe* and *L. sulphureus*, respectively. This removal was quite proportional to the TBP degradation, indicating a dehalogenation of the compound without the formation of another organo-brominated compound.

To determine TBP biodegradation in a natural substrate, *E.*

globulus sawdust was used. The fungus employed was *G. australe*, given the higher degree of degradation that reached in liquid culture. TBP biodegradation in sawdust had an approximated maximum of 23%. This could be due to that the wood is a natural substrate for fungi, becoming the main source of nutrients and limiting compound degradation (cometabolic conditions) (Fahr et al. 1999).

Chemical treatment

TBP degradation by Hamilton system shows 85% degradation after 1 hr and 95% degradation after 5 hrs and then remained constant (Figure 2). The mineralization percentage (TOC removal) and COD reached approximate values 50% and 48%, respectively. These results showed the limited efficiency of the Hamilton system in the mineralization of TBP. This is a common result in advanced oxidation systems, which generally must be combined with biological treatment (Vidal et al. 2000; Gotvajn and Zagorc-Konèan, 2005). The toxicity expressed as EC₅₀ (effective concentration in percentage terms) of the no-treated compound was 26%, which represents a concentration of around 15 mg L⁻¹, not changing with further treatment (Table 1).

Chemical-biological combined treatment

Chemical-biological treatment presented 100% degradation in 6 days. The toxicity of pre-oxidated samples treated with *G. australe* and *L. sulphureus* showed a reduction of 30% and 34%, respectively. The sample treated with *G. trabeum* presented an increase in toxicity of almost 50%, which could be due to compounds produced by this fungus or/and to hydroxylation products of the partial degradation of TBP (Kamada et al. 2002; Newcombe et al. 2002).

Table 1. Toxicity of TBP samples after chemical and chemical-biological combined treatment.

Samples	EC ₅₀ %
Control	26.4 ± 0.9
1,2-DHB/ Fe ³⁺ /H ₂ O ₂	26.2 ± 0.3
(1,2-DHB / Fe ³⁺ /H ₂ O ₂)/ <i>G. australe</i>	36 ± 3
(1,2-DHB / Fe ³⁺ /H ₂ O ₂)/ <i>G. trabeum</i>	15 ± 1
(1,2-DHB / Fe ³⁺ /H ₂ O ₂)/ <i>L. sulphureus</i>	40 ± 1

CONCLUDING REMARKS

TBP is a compound resistant to the aerobic biodegradation, but one that can be degraded by wood-degrading fungi. *G. australe* and *G. trabeum* were most efficient than *L. sulphureus* in this bioremediation process, presenting great ability to degrade the compound and remove organic bromine. The Hamilton system reached a high degradation percentage, mineralization and moderate COD reduction. No toxicity increase was observed, which is a favourable

factor compared with many advanced oxidation processes, in which the degradation products present more toxicity than the original compound. To optimize mineralization, the chemical system could be combined with the fungi biological treatment. This study demonstrated that the combined treatment provides a quite efficient process for the treatment of TBP residuals.

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