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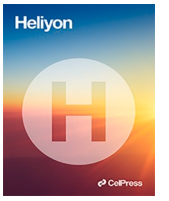

Removal and detoxification of pentahalogenated phenols using a photocatalytically induced enzymatic process

A. Meizler [a,*], N.A. Porter [b], F.A. Roddick [c]

[a] Department of Post-Graduate, Hong Bang International University, 215 Điện Biên Phủ, P.15, Q. Bình Thạnh, Ho Chi Minh City, 700000, Viet Nam
[b] School of Science, RMIT University, GPO Box 2476, Melbourne, Victoria, 3001, Australia
[c] School of Engineering, RMIT University, GPO Box 2476, Melbourne, Victoria, 3001, Australia

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ABSTRACT

Poly-halogenated phenols generated from a range of industrial processes can find their way into rivers and ground water. Here we report on a potential treatment for reducing the toxicity of these aqueous pollutants using two highly toxic penta-halogenated phenols (pentachlorophenol (PCP) and pentabromophenol (PBP)) as surrogates. Solutions were passed through a glass column packed with a silica support fused with titanium dioxide (TiO_2) and horseradish peroxidase (HRP) immobilized on its TiO_2/glass surface (HRP-T_{glass}). TiO_2 photocatalysis was activated through irradiation with UVB (320 nm) which in turn activated the HRP.

Two operational flow rates (0.5 and 1.25 mL min^{-1}; hydraulic retention times (HRTs) of 20 and 8 min, respectively), tested the effect of retention time on the extent of degradation and reduction in toxicity of the treated effluent. Microtox® was used to measure the toxicity of the substrate and its by-products at both flow rates. At the highest flow rate, dehalogenation was limited (removal of 37 % chlorine and 22 % bromine) and the toxicity of the reaction products increased. At the lowest flow rate, the longer exposure time resulted in approximately 97 % and 96 % transformation of PCP and PBP, respectively, a greater degree of dehalogenation (removal of 65 % chlorine and 70 % bromine) and a substantial decrease in toxicity of the treated solutions. The higher toxicity of effluent from the higher flow rate was attributed to the initial degradation products being more toxic than the substrates. With a longer HRT, these were then further broken down to less toxic products.

Additional toxicity tests (*Hydra hexactinella (*Hydra*)* and Chinese Hamster Ovary (CHO) cell toxicity were conducted on the effluent from the lowest flow rate. Both were less sensitive than the Microtox test, with *Hydra* proving more sensitive than CHO.

The novelty of this work is the toxicity risk assessment of the products resulting from the use of a spatially separated immobilized enzyme and photooxidation system. The system was robust and showed no decrease in treatment efficacy over 10 h.

1. Introduction

As the world population has grown, so has the volume of wastewater being generated. For example, in China alone, the volume of wastewater treated increased by 40 billion m^3 from 1990 to 2010 [1]. Growing industrial activity is increasing the load on wastewater

* Corresponding author.
E-mail address: sales@swarm-link.com (A. Meizler).

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treatment facilities [2,3]. However, government regulations and monitoring procedures do not always keep up to speed with current waste and sewage issues. This was highlighted by China's ban on processing foreign solid waste which left many countries, including Australia, with a significant problem for their recycling schemes [4].

Compounds such as poly-halogenated phenols are manufactured for use in a range of applications. Compounds such as pentachlorophenol (PCP, used in wood preservation [5]) and pentabromophenol (PBP, one of a large group of brominated phenols used as a flame retardant [6]) are part of this group. The Material Safety Data Sheets for pentachlorophenol and pentabromophenol note their aquatic toxicity and moderate bioconcentration factors as up to 10^4 for PCP and 10^2 for PBP [7,8]. However, the brominated flame retardants are similarly documented regarding their human health concerns as endocrine disruptors [9], and the chlorinated phenols are documented as affecting the nervous system [10], and with long exposure potentially causing cancer [11,12].

Pentachlorophenol is a serious concern regarding bioaccumulation due to its presence in meat and fish products [13]. Results obtained by Hong et al. [14] from the Pearl River Delta, China, showed levels of 30 ng g^{-1} of pentachlorophenol in the sediment of the local drinking water source, and in human breast milk to give a daily intake of up to 10 μg $infant^{-1}{\cdot}day^{-1}$. Since the US signed the Stockholm Convention in 2015 banning pentachlorophenol there are still reports of its use as a wood preservation for utility poles [15]. To complicate the situation several researchers have described the treated utility poles as having a longer lifetime than concrete or steel options [16,17].

With the increasing incidence of detection of brominated flame retardants in wastewater in Germany, Canada and Vietnam [18–22], it is becoming apparent that conventional treatment is not completely effective.

The current two most effective treatment methods for flame retardants and wood preservatives are coagulation and precipitation through the alum/Fenton process, and adsorption on ion exchange matrices [23]. Due to the high cost of these processes, most plants still use a cheaper biological option; however, this is not wholly effective as shown by Kumar and Chandra [24]. They recorded the levels of total phenols before (29 mg L^{-1}) and after (6 mg L^{-1}) biological treatment and pH adjustment of wastewater from a pulp and paper factory. They indicated that the plant produces 80,000 imperial gallons day^{-1} of toxic wastewater, equivalent to 0.8 tons of phenols discharged per year, and by-products such as polychlorinated phenols from the bleaching process were also found in the wastewater stream (levels were not mentioned).

Alternative processes using different combinations of anaerobic and aerobic processes and advanced oxidation (ozone, UV with peroxide) have been investigated throughout the years. A potential treatment process is to include the use of enzymes. Enzymes are effective natural catalysts with high specificity and high turnover properties compared to traditional microbially-mediated biological treatment which requires lag/acclimatisation time. They can target pollutants of concern and treat them without the need to separate them from the medium [25]. However, the high operation costs such as supply, storage, recovery and delivery make the commercial utilization of enzymes a challenge [26].

Enzymes can be used directly, immobilized on a medium (glass beads, filter, surface coating or encapsulated), and with the addition of bacteria or fungi to increase performance. All these enzyme preparations have a degree of success, but most (such as horseradish peroxidase) require a feed of co-factors such as H_2O_2 and pH adjustment. Furthermore, there is a gradual reduction in activity due to deactivation of the enzyme over time.

Horseradish peroxidase (HRP) has a specific reaction mechanism that utilizes H_2O_2 to convert phenolic compounds into radicals which in turn interact with each other to form high mass polymers which can be precipitated [27]. In addition, in the presence of H^+ the activated enzyme can convert the attached halogen into radical form which then becomes an anion [28–30].

UV photolysis, either direct or through water or other oxygenated groups as an advanced oxidation process, may also be utilized and leads to a variety of degradation products, including free anions [31,32].

López et al. [33] reviewed the effectiveness of treating phenolic compounds via a combination of photocatalysis and biocatalysis using light to indirectly fuel the reaction. Using HRP-T_{glass} we also reported [34,35] the benefits of this combination.

a) the provision of *in situ* H_2O_2 generation (which in turn is utilized as hydroxyl radicals) from the photocatalysis of water on the surface of the TiO_2;
b) polymerization of the phenol via the enzymatic reaction;
c) prevention of the precipitation of the polymerized products on the support due to hydrophilic repulsion by the TiO_2 of any hydrophobic material formed, thus preventing deactivation; and
d) provision of adequate protection to the enzyme from change in environmental conditions.

This approach of using a photocatalytic reaction to induce an enzymatic reaction may have the potential to improve the wastewater treatment process. It could also reduce the overall cost by replacing the hydrogen peroxide co-factor with a light source and by including immobilization of the enzyme on a solid surface to increase its recovery and stability, thus increasing its lifespan [36].

In Meizler et al. [34] we described immobilized horseradish peroxidase (HRP) on a novel glass support containing photocatalytic titanium dioxide (TiO_2) on which the enzyme and TiO_2 were spatially separated. That combination yielded excellent results: polymerization/precipitation of 4-bromophenol (95 % TOC reduction and 70 % bromide formed at optimal conditions) in an aqueous solution. The aim of this paper is to use the same process to investigate the efficacy of HRP/T_{glass} treatment to reduce the toxicity of a mixture of PCP and PBP.

2. Methods and materials

2.1. Chemicals

All chemicals used were of reagent grade or higher. Acetonitrile and methanol were purchased from Honeywell (Australia). Trifluoroacetic acid (TFA), pentachlorophenol (PCP) and pentabromophenol (PBP) were purchased from Sigma (Australia). Natural water was obtained from West Barwon Reservoir, Victoria.

2.2. HRP-tglass -packed column

The system (Fig. 1) comprised an empty column (25 mL) to regulate the flow followed by a 10 mL column containing 2.5 g HRP-T_{glass} particles constructed according to our previous paper [35]. The enzyme was linked to the silica matrix of the support via ethoxysilane ligands, but not to the TiO_2, and thus were spatially separated. The spatial topography of the particles was confirmed using Environmental Scanning Electron Microscopy.

The HRP-T_{glass} particles (125–150 μm diameter) contained titanium dioxide ($TiO_{2)}$ comprising 30 % anatase and 70 % rutile as determined by X-ray diffraction as the HRP support. They had an average surface area (BET) of 9.4 $m^2\ g^{-1}$ and average pore size of 70 Å. The activity of the HRP-T_{glass} was 0.25 U pyrogallol g^{-1} measured using a modified pyrogallol activity test [34].

The UVB lamp ('TL' 20 W/12, 320 nm) irradiated the entire chamber during the experimental run. The intensity of UV irradiation (460 μW cm^{-2}) was measured at the surface of the column with a dosimeter (IL1400A, InternationalLight®, Singapore). The packed bed system was fed with PCP and PBP solutions. The feed was prepared in water from West Barwon Reservoir, Victoria (pH 7.2) and fed to the column in a single pass at two flow rates: 0.5 and 1.25 mL min^{-1} which were equivalent to hydraulic retention times (HRTs) of 20 and 8 min, respectively.

The system illustrated in Fig. 1 was fed the compositions given in Table 1. Samples for transformation and dehalogenation were collected periodically. For the toxicity and electrospray ionization (ESI) analyses, samples were taken when the reaction had plateaued. For the control run (UVB without HRP), 300 mL was passed through the column and the pooled effluent was analysed.

2.3. Characterization of products by liquid chromatography/mass spectrometry (LC/MS)

The products of reaction were characterized by 'ESI using a Quattro Ultima Micromass LC/MS. The analysis was conducted using a direct feed of solution at a rate of 10 μL min^{-1}, with settings for negative ions at a capillary energy of 2.5 kV and cone energy of 80 V in MS scan mode. The source temperature was 120 °C and desolvation temperature was 150 °C. Nitrogen was used as the desolvation gas at 550 L h^{-1}, and Micromass MassLynx 3.5 software was used for data analysis.

2.4. High performance liquid chromatography (HPLC)

The extent of oxidation of pentachlorophenol and pentabromophenol was determined from the concentration of the remaining substrate using HPLC (reverse phase chromatography) Gilson instrumentation consisting of a Model GX-281 solvent-delivery system with detection using a Model 156 UV–vis detector at 240 nm and a Lichrospher 100 RP-18 column (250 × 5 mm i.d., 5 μm (Merck, Germany)). The mobile phase consisted of component A (30 % v/v distilled water, 35 % v/v methanol, 35 % v/v acetonitrile in 1 mM TFA) and component B (10 % v/v distilled water, 45 % v/v methanol, 45 % v/v acetonitrile in 1 mM TFA) in the following program: initially 100 % A; linear gradient over 10 min to 100 % B; held isocratically at 100 % B for 5 min; linear gradient to 100 % A over 6 min; held isocratically at 100 % A for 8 min. The flow rate was maintained at 1.5 mL min^{-1} at ambient temperature.

2.5. Determination of anion concentration in solution

The extent of dehalogenation was determined by measuring anion concentration using an ion conductivity chromatograph (Dionex, USA). The anion exchange column (MetroSEP anion dual column 15 cm; 3 mm i.d., 10 μm) was run isocratically using 2.4 mM sodium bicarbonate, 2.5 mM sodium carbonate and 2 % (v/v) acetone as eluent at 1.0 mL min^{-1} and 1430 psi.

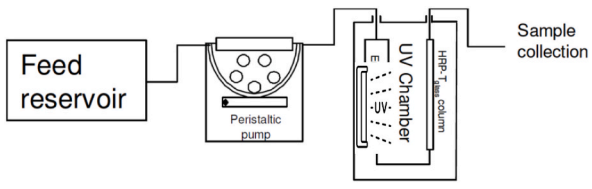


Fig. 1. Schematic representation of the HRP-T_{glass} packed column under UV radiation.

Table 1
Initial concentration of polyhalogenated phenol substrates[a].

Substrate	PCP	PBP	PCP plus PBP
High concentration (concentration to demonstrate the capacity of the system)			PCP 8 mg L^{-1} PBP 10 mg L^{-1}
Low concentration. (concentration required to elicit an effect in the test organisms)	0.67 mg L^{-1}	0.89 mg L^{-1}	PCP 0.67 mg L^{-1} PBP 0.89 mg L^{-1}

[a] PCP concentrations measured from Brega wastewater stream, Libya, in 2013 showed levels that ranged from 41 μg L^{-1} to 182 μg L^{-1} [37].

2.6. Toxicity tests

The toxicity of the effluent prior to and post treatment of the high concentration mixture was determined by the Microtox® test for both flow rates and rated according to the ranking scheme of Coleman and Qureshi [38]. In this method, the test organism is subjected to a fixed concentration of the substance under investigation. The percentage of test species having no apparent effect is the variable measured (<25 % = highly toxic; 25–50 % = moderately toxic; 51–75 % = toxic; >75 % = slightly toxic; ≈100 % = non-toxic).

The toxicity of the effluent prior to and post treatment of the low concentration solutions (PCP, PBP and combined) at the low flow rate were determined with three types of toxicity tests: Microtox®, *Hydra hexactinella* (Hydra) and Chinese Hamster Ovary (CHO) cell toxicity.

1. The Microtox® test determines the degree of toxicity by the extent to which the luminescence emitted from the bioluminescent bacterium *Aliivibrio fischeri* is reduced. The Microtox® tests were conducted by Geotechnical Services Pty Ltd.
2. *Hydra hexactinella* is a freshwater microinvertebrate ubiquitous in freshwater environments. When exposed to toxic substances adult *Hydra* undergo major morphological changes. The clubbed tentacle stage was selected as the sub-lethal endpoint, and the tulip phase was selected as the lethal endpoint. All calculated values of toxicity for the *Hydra* test were obtained using the Trimmed Spearman-Karber method [39]. The *Hydra* toxicity tests were conducted at the Ecotoxicology Laboratory at RMIT University.
3. CHO cells are introduced to Thiazolyl Blue Tetrazolium Blue (3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) which reduces the yellowish solution via metabolically active cells to purple formazan. The rate of formazan production, which is directly proportional to the number of viable cells, was measured at 560 nm in a microplate reader. IC_{50} values were calculated using probit vs log concentration on SPSS. The CHO toxicity tests were carried out at the Ecotoxicology Laboratory at RMIT University.

3. Results

To estimate the system efficiency, the high concentration mixture of PCP and PBP was fed to the column in a single pass. At the low flow rate (0.5 mL min^{-1}) transformation of both species plateaued at ≈97 % after 3 h (90 mL treated), whereas at the higher flow rate (1.25 mL min^{-1}) the system plateaued at ≈60 % transformation of the PCP and PBP within an hour (75 mL treated) as shown in Fig. 2.

The concentration of the chloride and bromide released reached a plateau corresponding with the respective substrate transformation (Fig. 2). At the low flow rate, chloride concentration in the effluent plateaued at 66 % and the bromide at 72 % of the available corresponding halogen (Fig. 2a), while at the higher flow rate, the chloride averaged 30 % and the bromide averaged 20 % of the available halogen (Fig. 2b) from the transformed substrate (single run was analysed).

The transformation of PCP compares well with the results obtained by Tolardo et al. [40]. They reported partial transformation of PCP (1 mg L^{-1}) using free soybean peroxidase and hydrogen peroxide at pH 5–7; however, only with the addition of Fe(II) were they able to achieve complete transformation.

From a comparison of the molar ratio of halogen released to the theoretically available halogens in the transformed PCP and PBP, it appears that the chlorine and bromine are more easily removed at a lower flow rate, as an average of 3 of the available 5 chlorine/

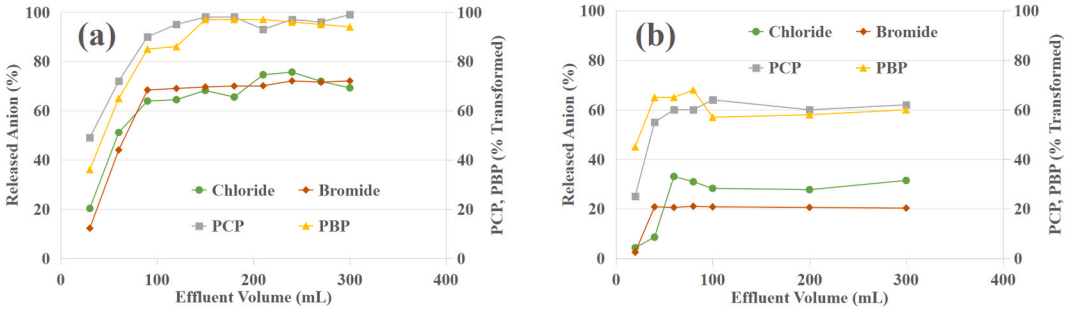


Fig. 2. Extent of transformation and dehalogenation of PCP and PBP (8 and 10 mg L^{-1}, respectively) by UVB/HRP-T_{glass} in a single pass (a) flow rate 0.5 mL min^{-1} (b) flow rate 1.25 mL min^{-1}.

bromine atoms occur in the effluent. However, at the higher flow rate (shorter UVB exposure time) both halogens proved harder to detach.

For the control run (no HRP; 0.5 mL min^{-1} HRT of 20 min) the transformation results were 69 % for PCP and 51 % for PBP and the formation of available chloride and bromide was only 9 % and 8 %, respectively. These results show that only 1 out of 5 halogens was removed. This is a low yield compared to the runs where the system included HRP which were able to remove 3 halogens out of the available 5.

The release of halogens here compares well with other treatments such ozonation. Dar et al. [41] were able to remove only 2 bromine atoms per molecule from pentabromophenol while Javier-Benitez et al. [42] obtained single chlorine atom release per molecule from pentachlorophenol using either ozonation or UV photodegradation.

At face value, these numbers suggest that UVB is able to utilize the photocatalytic ability of TiO_2 to form Cl and Br radicals, but the addition of HRP increases the conversion of the halogens into radicals that result in the formation of anions as discussed in the introduction [28]. Change in pH was not considered an issue due to the initial low levels of substrate. There are numerous articles that utilize TiO_2 + UVB to treat PCP, some with great success, however, a recent report by Ma et al. [43] showed that the intermediates are even more toxic than the original substrate. This means that even if the original substrate has been transformed, longer treatment is needed to also remove the intermediates.

The ESI mass spectrum of the polymerized and dehalogenated products of PCP and PBP in the effluent is presented in Fig. 3 and some of the compounds were identified.

On completion of the run, the treated mixture of PCP and PBP was analysed for the relative peak intensities of the isotopic clusters of ions and their fragmentation patterns, knowing that the original compounds lose chlorine and bromine. Given the presence of free chloride and bromide, it is expected that the mass spectrum will contain various forms of PCP and PBP which have been partially to completely stripped of their halogens. Analysis of the products resulting from treatment of the lower concentrations of the halogenated phenols was unsuccessful due to low signal to noise ratio masking the data.

Taking into consideration the ESI and substrate transformation data, we propose that the UV photocatalysis leads to photo reduction (via the conduction band of the TiO_2) releasing radical ions from the substrate, and photooxidation (via the valence band of the TiO_2) which promotes conversion of water to hydroxyl radicals. The hydroxyl radicals drive the peroxidase reaction to yield oligomers. Osborne et al. [28], have reported that another reaction product, halogen radical, is formed.

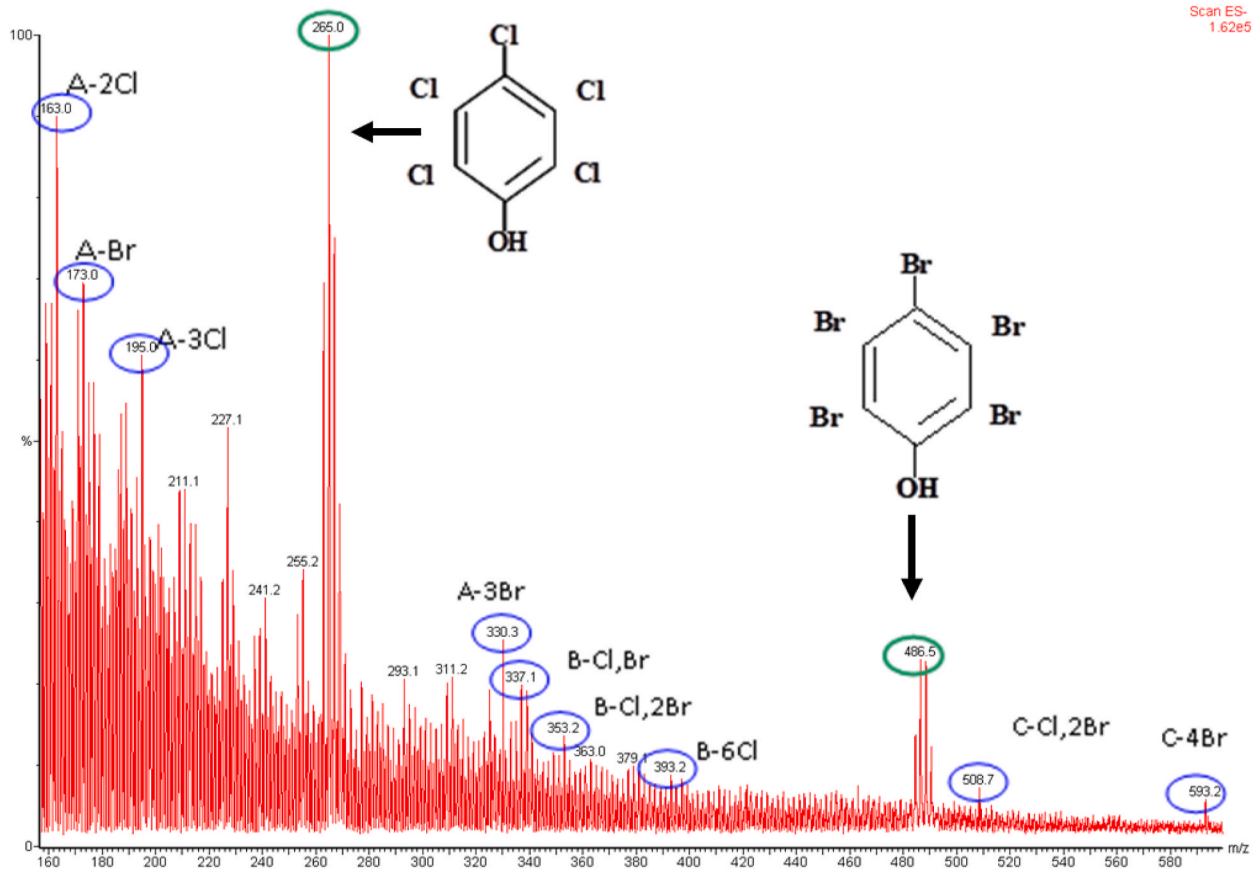
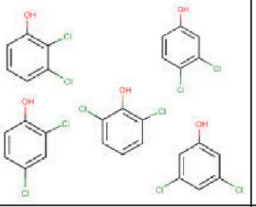
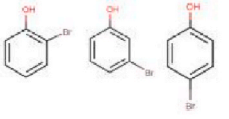
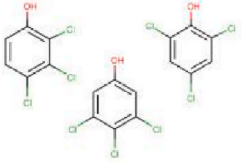
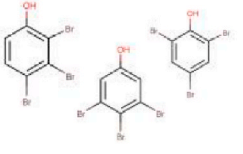
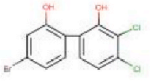
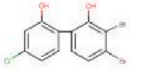
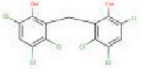


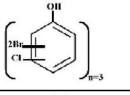
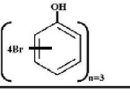
Fig. 3. ESI mass spectrum obtained from the mixture of PCP and PBP (8 mg L^{-1} and 10 mg L^{-1}, respectively) treated at the lowest flow rate. A- monomers; B- dimers; C- trimers.

Table 2
The distribution of molecular masses indicated from the ESI Spectrum in Fig. 3 and their EC_{50} values (obtained from the Hazardous Substances Data Bank (HSDB)).

Species	Indicated compound and chemical formula	Structure	Molecular Weight	EC50 (μgL^{-1})
A- Monomers	Dichlorophenol (C6H4Cl2O)		163	1700-4100
	Bromophenol (C6H5BrO)		173	13000-11000
	Trichlorophenol (C6H3Cl3O)		195	890-1700
	Tribromophenol (C6H3Br3O)		330	1310

Species	Indicated compound and chemical formula	Structure	Molecular Weight	EC50 (μgL^{-1})
Substrate	Pentabromophenol (C6Br5OH)		489	79800-108000
Substrate	Pentachlorophenol (C6Cl5OH)		266	130-81000

Species	Indicated compound and chemical formula	Structure	Molecular Weight	EC50 (μgL^{-1})
B- Dimers	Dichloro-bromophenolic dimer (C12H7Cl2O2Br)		337	NA
	Dibromo-chlorophenolic dimer (C12H7Br2O2Cl)		379	NA
	Hexachlorophene (C13H6Cl6O2)		407	198

Species	Indicated compound and chemical formula	Structure	Molecular Weight	EC50 (μgL^{-1})
C- Trimers	Dibromo-chlorophenolic trimer (C18H8O3Br2Cl)		509	NA
	Tetrabromo-phenolic trimer (C18H9Br4O3)		593	NA

The halogen radical can either form free ions or attack organic compounds. However, it is more likely to attack the already dehalogenated products rather than the penta-halogenated phenol in which all the sites are occupied. This explains the formation of mixed brominated and chlorinated oligomers seen in the ESI spectrum (Fig. 3).

These data sets and knowledge of the initial substrate fragmentation mass and form allowed identification of dehalogenated derivatives from the oxidation of the parent PCP (MW 265.0) and PBP (MW 486.5). The aquatic toxicities of the identified by-products, which varied with aquatic organism, were then compared (Table 2). The EC_{50} values (concentration that gives half maximal response) were obtained from the Hazardous Substances Data Bank (HSDB) and more information can be obtained from OPP Pesticide Ecotoxicity Database (https://ecotox.ipmcenters.org/index.cfm?menuid=5).

Several researchers reported that the activity of immobilized peroxidase during conversion of phenolic compounds to polymers gradually decreased with time [44,45] due to precipitation of the polymer product, and the larger the polymer the greater its hydrophobicity and so the greater the likelihood of its precipitation from aqueous media. That was not the case here; the data presented in Fig. 3 and Table 2 were acquired from the aqueous solution, i.e., soluble products, not an extract of potential precipitated products.

These precipitated by-products may be a concern due to the high level of halogenated phenol conversion into oligomers with relatively low halogen count. The larger the oligomer/polymer, the higher the halogen count and the higher the toxicity [46,47].

3.1. *Toxicity of treated PCP and PBP solutions according to Microtox® test*

Microtox® testing was conducted on the high concentration PCP and PBP mixture prior to and post treatment at the low flow rate, all samples were found to be extremely toxic. From the values of the reaction products (Table 2), it appears that the toxicity of some of the by-products exceeded that of the original solution. Given that the EC_{50} of PCP, the most toxic of the two compounds, was 0.13 mg L^{-1} it was clear that the concentration in the initial experiments was too high (PCP 8 mg L^{-1}; PBP 10 mg L^{-1}). The experiment was repeated using a lower concentration mixture containing 0.67 mg L^{-1} PCP and 0.89 mg L^{-1} PBP run at both flow rates (Fig. 4). This demonstrated that the UVB/HRP/T_{glass} system was able to treat and detoxify the halogenated phenols. The initial mixture toxicity falls within the EC_{50} parameter of the dominant PCP levels.

Microtox® testing revealed that the toxicity of the PCP and PBP mixture treated at the higher flow rate was greater than for the untreated substrate (reduction in bioluminescence 73 % cf. 54 %), whereas at the lower flow rate the reduction in bioluminescence of the treated mixture was less (35 %), indicating a reduction in the toxicity of the original substrate. Hence the reduced toxicity (Fig. 4) is associated with the greater dehalogenation which occurred at the low flow rate (Fig. 2), and thus higher exposure time. Similar results were obtained by Dar et al. [41] who treated pentabromophenol with ozonation. They reported that their by-products contained dimers that lost 1–2 bromines from the original PBP which resulted in higher toxicity than the original. They concluded that high halogen content of a dimer/trimer by-product will elevate the toxicity of the reaction mixture and thus the compound. We suggest that it is necessary for the UVB/HRP-T_{glass} system to produce at least 70 % dehalogenation of the halogenated phenols, especially when there is a mixture of different halogens, as there is a tendency for the toxicity to increase with less than this.

We also investigated other by-products which may influence the toxicity, such as the hydroxyl anion and peroxide. However, these compounds were found to be below detection level (lowest detection level for H_2O_2 is 0.38 mg L^{-1} and LC_{50} (concentration lethal to half of test species) is 5 g kg^{-1}) and to degrade further in the presence of a reductive substrate (e.g., phenolic compound) [35]. Other by-products with the potential to influence toxicity were chloride and bromide (with sodium) which have an oral rat LD_{50} of more than 3 g kg^{-1}; bromide is considered a mutagen, and long-term use of bromide-based sedatives can result in bromism, a psychiatric disorder due to impaired neuronal transmission [48]. The levels of Cl^- and Br^- were found to be below the limit of detection (0.09 mg L^{-1} for Cl^- and 0.2 mg L^{-1} for Br^-).

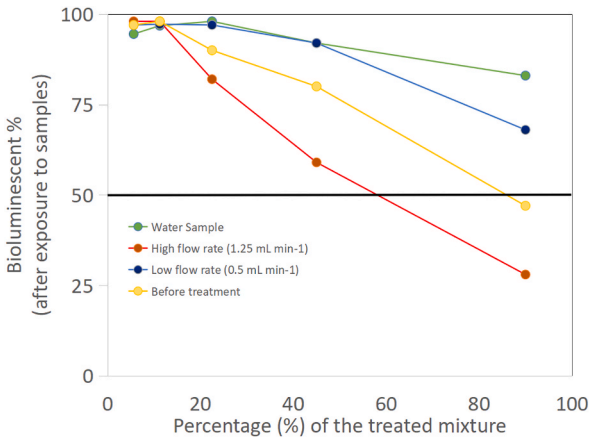


Fig. 4. Results for Microtox® tests performed on reservoir water spiked with a mixture of PCP and PBP.

3.2. Toxicity of lower concentration PCP and PBP solutions

The transformation of PCP, PBP and their mixture (0.67 and 0.89 mg L^{-1}, respectively) was carried out at the low flow rate of 0.5 mL min^{-1} and resulted in greater than ≈97 % transformation and greater than ≈70 % chloride and bromide release. The toxicity of the solutions before and after treatment was evaluated in terms of Microtox®, *Hydra* and CHO LC_{50} values (Fig. 5).

Overall, the toxicity of all the solutions was significantly reduced after treatment. The only exception was the mixture of PCP and PBP which had a slight reduction in toxicity as seen in the results of the 24 h exposure in the Microtox® test. This is expected due to the combined concentrations of PCP and PBP together in the mixture and the different toxicity of mixed halogenated oligomers.

The LC_{50} and EC_{50} values for the *Hydra* tests show that the toxicity of the effluent was reduced for all the solutions, however not to the extent indicated in the Microtox® tests. Interestingly, Castillo et al. [49] and Arias-Andrés et al. [50] stated that aquatic organisms such as *Daphnia* and *Hydra* are the most sensitive indicators for measuring aquatic life mortality. The results are very satisfactory for the mixture of PCP and PBP as the toxicity after treatment is greatly reduced. CHO cell toxicity tests are normally used to investigate the impact of drugs on mammals. In these results the LC_{50} values were close, tending to show little impact of the treatment and suggesting that the test is not sensitive to the toxicants under investigation here. When compared with the CHO toxicity test results reported in the National Toxicology Program 1999 report [12], doses higher than 80 μg mL^{-1} of PCP, i.e., markedly greater than those used here, led to positive chromosomal aberrations.

When Sellami et al. [51], Wang et al. [52] and Feng et al. [53] reported toxicity reduction when phenol-based compounds were either oxidized or reduced, they also reported that the extent of treatment was dependent on the pH. It should be noted that pH is important as the cost of adjustment of pH is high. The pH must be appropriately adjusted prior to discharge to water bodies (river, pond or ocean) to avoid negatively affecting aquatic life. We reported [35] that the UVB/HRP-T_{glass} system enabled maximum transformation of 4-bromophenol over the wide pH range of 4–11, and thus treatment at close to neutral pH which precludes the need for pH adjustment of treated municipal wastewater (usually pH 7–8.5) prior to its discharge to local water bodies. As a result, ≈97 % transformation of halophenols is possible with this system without the need to employ extremes of pH or temperature.

As the treated mixture products remained in the solution, the main contributor to toxicity is the degree of halogenation. This is supported by a study by Wagner and Nicell [54] who found that HRP treatment of phenolic solutions (phenol, 2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol and 2-methylphenol) led to doubling of toxicity. However, they reported that they did achieve detoxification by using UV irradiation for 5 min regardless of whether H_2O_2 was present or not. A more recent study by Prasse et al. [55] adds to this finding; they found that the reaction of phenols with $^{\bullet}OH$ and UV light produce oxoenal and enedial which in low concentration are highly toxic via high nucleophilic addition reactivity with DNA and RNA. This is consistent with the work of Bryant and Schultz [56] who discovered that the formation of intermediate metabolites from PCP by microbial oxidation resulted in enhanced toxicity.

4. Conclusion

It is evident that pollutants are present in the environment and published data are showing an increase in persistent halogenated phenols. In this work, reaction of an immobilized enzyme initialised by photocatalysis (UV irradiation) demonstrated a very successful treatment of the representative pollutants PCP and PBP. Flow rate was controlled in a simple continuous column system to achieve optimal transformation without the need for the addition of any co-enzymes or modifiers. The robustness of the system was demonstrated by consistent performance for 10 h.

The treatment resulted in transformation of up to ≈97 % of PCP and 96 % of PBP, and the effluent contained chloride and bromide in addition to small chain oligomers, some of which were halogenated. We observed that the degree of dehalogenation played a pivotal role in the extent of overall toxicity. However, the decrease in toxicity following dehalogenation and the transformation of the substrates were not directly related. We have concluded that longer treatment decreased toxicity because the system we developed broke down the toxic intermediate products.

The three different toxicity tests used (bacterial, freshwater invertebrate and mammalian), gave differing results. Of these, the Microtox test (bacterial) was the most sensitive. The *Hydra* test (freshwater invertebrate) was moderately sensitive, while the CHO test showed minimal to no change.

The results of the present work clearly show the potential of continuous removal of soluble phenolic pollutants from water and wastewater streams by an immobilized physico/biological approach.

There is potential for the water and wastewater industries to benefit from this type of treatment system as it is simple and easy to operate. Future scale-up of this system must take into consideration the operational parameters to ensure complete treatment of any halogenated phenols.

CRediT authorship contribution statement

A. Meizler: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **N.A. Porter:** Supervision, Writing – review & editing. **F.A. Roddick:** Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

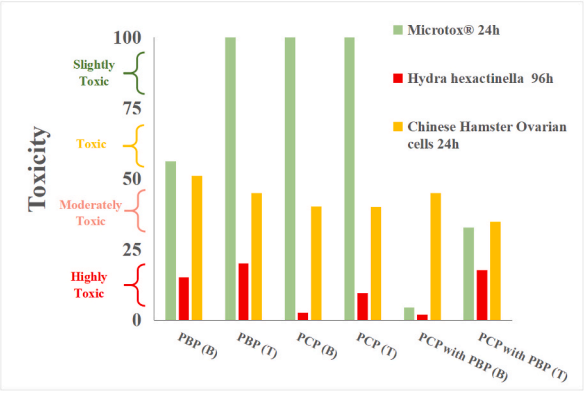


Fig. 5. A comparison of half-maximal concentration results for Microtox®, *Hydra* lethal and CHO cell inhibitory lethal (LC_{50}) tests performed on reservoir water spiked with PCP and PBP (0.67 and 0.89 mg L^{-1}, respectively) before treatment (B) and after treatment (T).

influence the work reported in this paper.

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