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TOXICITY STUDIES DURING THE DEGRADATION OF PENTACHLOROPHENOL BY OZONATION IN THE PRESENCE OF MnO_2/TiO_2

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ABSTRACT

Ozone is a strong oxidant used in the water treatment to remove organochloride compounds. Given that many processes of degradation generate chemical compounds that are more toxic than initial compounds, the development of optimized ozonation processes are required. In this study, pentachlorophenol (PCP) was used as a model of an organochlorine compound and the toxicity of its degradation products generated by both non-catalytic and catalytic ozonation processes were evaluated with a fresh-water *Daphnia magna* (ecotoxicity) and vegetables species *Lactuca sativa* and *Panicum millaceum* (Phytoxicity). The catalytic ozonation used MnO_2/TiO_2 as catalyst, which was characterized by X-ray diffraction analysis showing the presence of crystalline TiO_2 phases, rutile and anatase. The specific BET surface area of MnO_2/TiO_2 was 43 m^2/g. It was found that the use of ozone as an oxidant showed a first order degradation rate constant (k_{obs} = 0.5 ± 0.1 min^{-1}). The uncatalyzed reaction showed several reaction intermediates like mono- and polychlorinated phenols, and quinones. The oxidation of these compounds led to low molecular weight organic acids. From these results, we proposed a pathway of PCP degradation using ozone. The catalyzed reaction showed a more potent effect in reducing the toxicity of the treated solution. Unlike the use of only ozone which does not decrease the toxicity. It was found that the treatment with catalytic ozonation decreases the toxicity of the solutions.

Keywords: Pentachlorophenol (PCP); catalytic ozonation; MnO_2/TiO_2; toxicity assays; *Lactuca sativa*; *Panicum millaceum; Daphnia magna*.

1. INTRODUCTION

Pentachlorophenol (PCP) has been used extensively as a broad-spectrum herbicide, as well as pesticide and wood preservative world-wide.[1,2] PCP is toxic to both plants and animals; due to its high toxicity, PCP is ranked among the priority pollutant list of the US Environmental Protection Agency (EPA) in 1978, and it is classified as a Group B2, probable human carcinogen by the EPA.[3,4] The PCP has a moderate solubility in water (15 mg/L), a rather high octanol-water constant (log K_{OW} = 5.1) and very low concentration (0.1 µg/L), which can cause a deterioration of the ecosystem.[5] It is reported that the maximum concentration allowed should not exceed 0.055 mg/L in water surfaces.[6]

In Chile the manufacture, marketing and application of pesticides that contain PCP was prohibited according to the legislative decree Nº 78 in 2004, however remediation processes are needed to destroy the remaining compound. Incineration is a common method to dispose waste organic compounds, but this process generates polychlorinated compounds like dibenzo-*p*-dioxins (PCDDs)[7] and polychlorinated dibenzofurans (PCDFs) as by-products[8,9] which are as toxic, carcinogenic and environmentally persistent.[10,11] Therefore, it is of great importance to develop environmentally friendly techniques to eliminate PCP. Because of the toxic and recalcitrant nature of PCP, its degradation by traditional biological processes has not been satisfactory. So, it is highly recommended to seek removal methods for PCP degradation into less harmful intermediates, or even achieve its complete elimination by mineralization processes.

Due to various adverse effects of PCP, several physical and chemical techniques have been used to remove it from aqueous solutions. The use of advanced oxidation processes (AOP) in the treatment of industrial wastewater containing high concentrations of refractory compounds based on the generation of very reactive groups, particularly hydroxyl radicals have been carried out. Among these technologies, the O_3 process is more efficient in pollutant degradation and less harmful to most living organisms compared to other oxidizing reagents like H_2O_2 or chlorine.[12, 13, 14]

Ozone is a very strong oxidant, but single ozonation process performance is not strong enough to remove the organic matter by mineralization because some oxidative reactions are relatively slow and selective. During the process of ozonation, the concentration of the substrate vanishes, but this is not indicative of the mineralization of the reactant, and the different products generated as intermediate compounds may be even more toxic than the initial pollutant.[15,16] Nowadays, AOPs have been studied to provide greater ozonation efficiency, and catalytic ozonation is found to be more effective for the removal of several pollutants from aqueous solution.[17]

Among AOPs, heterogeneous catalytic ozonation has received much attention in water treatment[18] due to its high oxidizing potential,[19] and the improvement in their performance when using metal oxides, or metals on metal oxide supports (e.g. MnO_2, TiO_2, Al_2O_3, Cu-Al_2O_3, Cu-TiO_2, Ru-CeO_2, V-O/TiO_2, V-O/silica gel and TiO_2/Al_2O_3, Fe_2O_3/Al_2O_3).[20,21,22,23]

During degradation of organic pollutants, the removal of recalcitrant compounds generated is difficult. Our group reported the removal of recalcitrant compounds such as oxalic acid in aqueous solutions using a catalytic ozonation process with MnO_2/TiO_2.[24] However, these studies should be supported with toxicity studies.

In the present work, we present the study of degradation process PCP by ozonation in a batch reactor using MnO_2/TiO_2 as a catalyst. The effect of the pH solution, the relationship between the disappearance rate of the model compound, the generation of intermediates, and the toxicity of the medium during the reaction were also studied. The intermediates of these processes were identified by chromatographic techniques and their toxicity on biological systems was evaluated using *in vivo* tests on *Daphnia magna*, *Lactuca sativa* and *Panicum millaceum*.

2. MATERIAL AND METHODS

2.1 Reagents and chemicals

Pentachlorophenol 86% p.a. (Aldrich) was used without further purification, hydrogen peroxide reagent grade 30% (Merck), analytical grade reagents or calibration standard supplied by Merck or Mallinckrodt were used for HPLC measurements. All solutions used in the degradation studies were prepared using double distilled water. The catalyst was prepared using a commercial TiO_2 Degussa P25 and $Mn(NO_3)_2.4H_2O$ (Merck).

2.2 Catalyst

Catalyst MnO_2/TiO_2 was prepared using TiO_2 Degussa P25 as support with 1% w/w MnO_2, by impregnation at 35ºC with an aqueous $Mn(NO_3)_2$ solution using the incipient wetness method. The solid was dried at 120ºC for 12 h and calcined in air at 500ºC for 4 h.[25](Villaseñor, 2002). Its characterization was by evaluated specific surface area and porosity in an automatic Gemini 2370 Micromeritics system, from the N_2 adsorption isotherm at -196ºC in the relative pressure range of 0.05–0.995. X-ray analysis for the catalyst was made on a Bruker D8 Advance instrument with Linear LynxEye detector, Bragg-Brentane geometry, Cu λ = 1.5406 A, 40 KV - 30 mA power, and 0.1 mm fixed optics. Temperature Program Reduction (TPR) experiments were carried out

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in a TPR/TPD (Temperature Program Desorption) 2900 Micromeritics system provided with a thermal conductivity detector. The reducing gas was a mixture of 5% H_2/Ar (40 cm^3/min) and a heating rate of 10 K/min was used. The TPD of ammonia was carried out using an Ar flow of 50 cm^3/min as carrier gas. Ammonia pulses were dosed in order to saturate the catalyst surface at 100ºC; the sample was cooled to room temperature, and once the base line was restored the temperature was increased linearly (10 K/min) up to 500ºC.[24] The zero point charge (zpc) was obtained by measuring the zeta-potentials as a function of pH suspensions.[26] The measurements were carried out in a Zeta-Meter Inc. (Model ZM-77) using 20 mg of 2 μm catalyst particles ultrasonically suspended in 200 mL of $1x10^{-3}$ mol/L KCl solution.

2.3 Degradation experiments

The following processes were studied: UV processes (UV and UV/H_2O_2), ozonation processes (O_3, O_3/H_2O_2, O_3/UV), and catalytic ozonation. The degradation process was performed in a Pyrex glass reactor.[27] It was charged with 90 mL of aqueous solution of PCP with an initial concentration of 30 mg/L. The MnO_2/TiO_2 catalyst (2.2 g/L) was kept in suspension with magnetic stirring. The external chamber of the reactor was kept at 20ºC or 40ºC by recirculation of water. For the ozonation processes, the ozone was generated by an OZOCAV ozonizer[25] and fed at an oxygen flow of 50 mL/min, reaching an ozone concentration of 22.11 mg/L. The H_2O_2 was used at $1.5x10^{-4}$ mol/L for each reaction.

For the experiments performed under UV light, the solution were irradiated with a General Electric UV lamp (HR 250 DX 37/40, $\lambda \geq 254$ nm) across the quartz reactor windows. The lamp was placed at the top of the window 5 cm over the sample.[27] The reactions were ran at pH 5, 7 and 9.

2.4 Analytical methods

Prior to the analysis, the samples were extracted from the reactor at several reaction times (0-60 min) and filtered through 0.20 μm membrane (Millipore). The concentrations of PCP and its degradation intermediates were measured with an HPLC system coupled to a Perkin Elmer Series 200 chromatograph with a UV–VIS detector. All the intermediates were identified by HPLC by comparing the retention time of the standard solution and comparing the GC-MS with the mass spectra of the intermediate compound in the database of NIST (National Institute of Standards and Technology). The following conditions were used for aromatic compounds; a Merck-Chromolith Performance RP-18 column (4.6 mm×100 mm). The mobile phase was an acetonitrile:H_3PO_4 ($7x10^{-3}$ mol/L) mixture (40:60), with a flow rate of 1.5 mL/min, and a wavelength detection at 215 nm. The analyses of the acid compounds were made with a Transgenomic ORH-801 column (6.5 mm × 300 mm) with $5x10^{-3}$ mol/L sulfuric acid as eluent (0.8 mL/min) at 200 nm.

Evolution of CO_2 was monitored according to previously described method.[27] Chloride samples were quantified by a potentiometric method[29] using an ORION specific chloride electrode.

2.5 Ecotoxicity assay

The ecotoxicity assays were performed using *Daphnia magna* (microcrustacean) and two plant species, *Lactuca sativa* (lettuce) and *Panicum millaceum* (millet), as dicotyledon and monocotyledon representatives, respectively.[28,29,30]

Several colonies of *D. magna* were maintained at 20ºC. All the tests were performed in the dark at constant temperature of 20 ± 2ºC. Acute toxicity was assessed by recording the effects of the compounds tested on the motility of *D. magna*. Daphnids less than 24 h old were used for the assays. The effective media concentration (EC_{50}) was determined as the concentration estimated to immobilize 50% of the daphnids after 24 h and 48 h of exposure with the 30 mg/L PCP solutions; the data was analyzed with Probit model statistical method.[31,32] The toxicity of the samples was reported as percent of immobilized daphnides with respect to a control (water). To compare the effects with the initial PCP concentration, the tests were performed using a same sample volume, higher than EC_{50} of PCP (0.75 mg/L at 48 hours). In all the experiments, a 300 μL of degradation process samples and PCP solution (30 mg/L) was completed to 10 mL with water.

Lettuce (*Lactuca sativa*) and millet (*Panicum millaceum*) seeds were selected as phytotoxicity bioindicators because they germinate very quickly and sprouts show rapid growth. Ten seed per plants were placed on a filter paper inside a vial and 200 μL of undiluted degradation samples were added. After a period, growth 3 days of light and 3 days of darkness at 25ºC, stems and roots length were recorded and expressed as the percentage length with respect of control plants treated with water. The statistical significance of differences between groups was determined by one-way ANOVA (*p-value* ≤ 0.05) and compared with the control group by the Dunnett-test.[28,29,30]

3. RESULTS AND DISCUSSION

3.1 Uncatalyzed Reactions

PCP degradation at a concentration of 30 mg/L was studied across the time in the absence of the catalyst. The pH of the solution was adjusted to pH 5, pH 7 and pH 9 with a phosphate buffer, and the process was monitored at 20ºC and 40ºC. Assuming pseudo-first-order kinetics, the rate constant was between 0.4-0.5 ± 0.1 min^{-1} in the presence only of ozone at 20°C in all pH tested; at 40°C, a slight increase in the rate constant of 0.7 ± 0.1 min^{-1} was achieved. At 10 minutes of uncatalyzed ozonation reaction, 96 ± 2% degradation of PCP at both temperatures was reached.

The **Fig. 1** shows the degradation of PCP to pH7 at 20°C in the presence of O_3, O_3/UV/H_2O_2 and UV/H_2O_2. In order to optimize the reaction system, UV, H_2O_2 and combinations of both were included; remarkably, in the absence of ozone a slow degradation of PCP was observed, with rate constants between $2x10^{-3}$ - $6x10^{-3}$ min^{-1}. On the other hand, no significant impact on PCP decomposition at both temperatures was observed under these conditions. The purpose of introducing UV radiation in the ozonation processes was to yield more free radicals for the higher ozonation rate, as was found in the decomposition of polyethylene glycol by O_3 with UV radiation.[33] These small differences found in the rate reaction for PCP degradation with the addition of H_2O_2 or UV irradiation, indicate that O_3 is the main agent involved in the process.

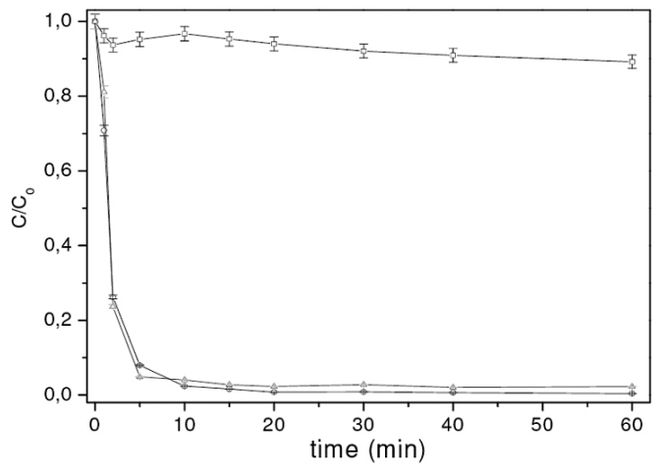


Figure 1: Degradation of PCP at pH 7 during 60 minutes in the presence of O_3 (Δ), O_3/UV/H_2O_2 (○) and UV/H_2O_2 (□).

In this study, chloride and CO_2 were found as mineralization products. The release of Cl^- after 60 min of ozonation was $4.2x10^{-4}$ mol/L at pH 9 and $2.2x10^{-4}$ mol/L at pH5. However, without O_3, the chloride production was lower. The formation of CO_2 was ~ $1.1x10^{-5}$ mol/L with O_3 and ~ $4x10^{-6}$ mol/L in the absence of ozone. The ozonation process led to an increased mineralization effect compared to the photochemical processes and oxidation by peroxide. The importance of O_3 in the PCP degradation is evident according to the reactions in the experiments, **Fig. 1**.

In water, ozone can oxidize contaminants by direct selective reactions, resulting in the addition to C=C bonds to or through a chain reaction mechanism that produces free hydroxyl radicals, which are stronger oxidants than molecular ozone.[34] The production of hydroxyl radicals by ozone in aqueous solution may be taken placed through chain reactions as shown in equations 1-4,[19] or by traces of other substances, such as transition metal cations, that leads to an indirect attack on organic compounds, which is faster than a direct attack by molecular ozone.[24]

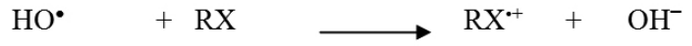
$$O_3 + H_2O \rightarrow 2\,HO^{\bullet} + O_2 \quad (1)$$

$$O_3 + OH^- \rightarrow O_2^{\bullet-} + HO_2^{\bullet} \quad (2)$$

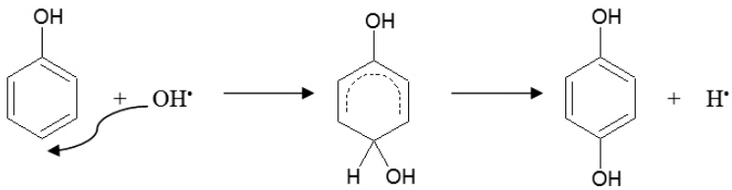
$$O_3 + HO^{\bullet} \rightarrow O_2 + HO_2^{\bullet} \quad (3)$$

$$O_3 + HO_2^{\bullet} \leftrightarrow 2\,O_2 + HO^{\bullet} \quad (4)$$

During the uncatalyzed ozonation process of PCP, intermediates formation, namely tetrachloro-1,2-hydroquinone (TCl-1,2-HQ) and tetrachloro-1,2-benzoquinone (TCl-1,2-BQ), at pH 5 and tetrachloro-1,4-hydroquinone (TCl-1,4-HQ) and tetrachloro-1,2-hydroquinone (TCl-1,4-BQ) at pH 9, were identified (**Fig. 2**). The major intermediate identified in the first minutes of uncatalyzed ozonation was TCl-1,2-HQ at pH 5, which may be produced as a result of the attack of either ozone or a hydroxyl radical on the carbon in *ortho* position of the PCP molecule. TCl-1,2-BQ is the oxidation product of TCl-1,2-HQ, which produces a reddish solution. At the same time, dehalogenation products such as phenols and mono-, di- and/or tri-chlorophenols were formed. It is likely that these later compounds are products of electron transfer reactions between the hydroxyl radical and PCP, which generate in the first instance cation radicals such as those shown in Schemes 1 and 2, as proposed previously by Legrini and co-workers.[35] These radicals may then react to produce the corresponding dehalogenation products. Moreover, the insertion of a hydroxyl group generates dehalogenation products, resorcinol and hydroquinone, as shown in the scheme 2. First, the dehalogenation of PCP produces tetrachlorocatechol, which also undergoes dehalogenation and addition of hydroxyl groups affording pyrogalol and catechol.

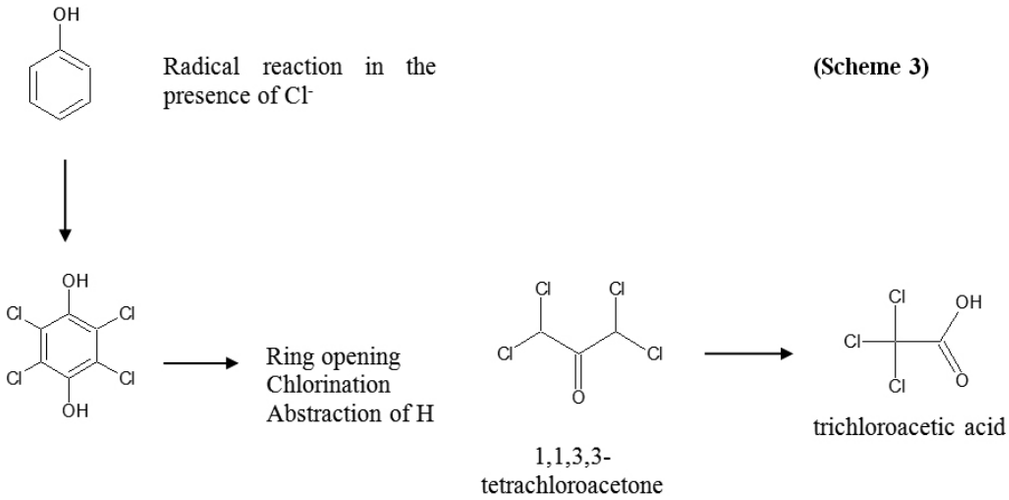


Sheme 1



Sheme 2

The subsequent oxidation of these compounds causes the breaking of the benzene ring and the formation of low molecular weight carboxylic acids such as maleic, oxalic, trichloroacetic and formic acids. The formation of trichloroacetic acid can be explained by the proposal made by Anipsitakis and co-workers[37] from 1,1,3,3-tetrachloroacetone (scheme 3):



Sheme 3

In addition to the formation of several quinones, the final steps in this oxidation process was the conversion of these carboxylic acids to complete mineralization products, carbon dioxide, chloride, and water.

It is noteworthy that TCl-1,2-BQ at pH 5 and 1,4-BQ at pH 9, were respectively formed as the main PCP degradation intermediates (**Fig. 3** and **4**). According to the pK_a of PCP, at pH 5 the phenolate ion reaches 67%, while at pH 9 it is 99.9% of the phenolate ion. The negative charge on the oxygen atom can hinder the attack of hydroxyl in an *ortho* position, favoring the addition in *para* position at pH 9. Under alkaline conditions, a greater degree of dehalogenation products like phenol, pyrogalol or resorcinol at higher concentrations than under acidic conditions was observed. The products of oxidation of these compounds at both experimental pH conditions included the formation of maleic, oxalic and formic acids, however at pH 9 there was generation of additional organic acids such as tartaric and malonic. For the mineralization products, chloride evolution was greater at basic pH than under acidic conditions. The percentage of dechlorination at 60 min of reaction reached 75% of the theorical value.

According to these results, we propose degradation pathways (**Fig. 3** and **4**) with the main intermediaries detected during the uncatalyzed ozonation process of PCP at pH 5 and pH 9.

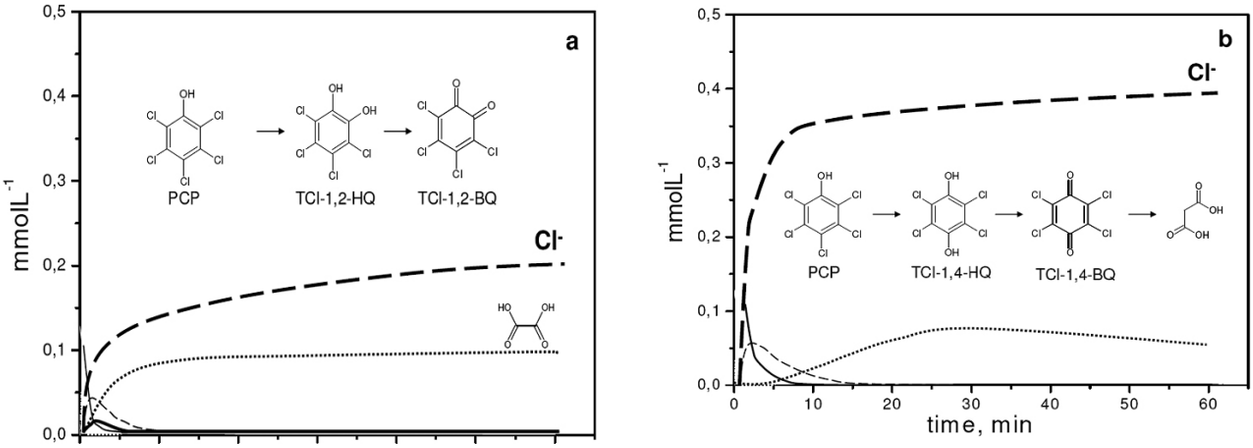


Figure 2: Degradation of PCP (—) at **(a)** pH 5 and **(b)** pH 9 with the evolution of Cl^- (– – – –) and the presence of the main products of degradation found under these conditions (TCl-HQ ---- and TCl-BQ ·····).

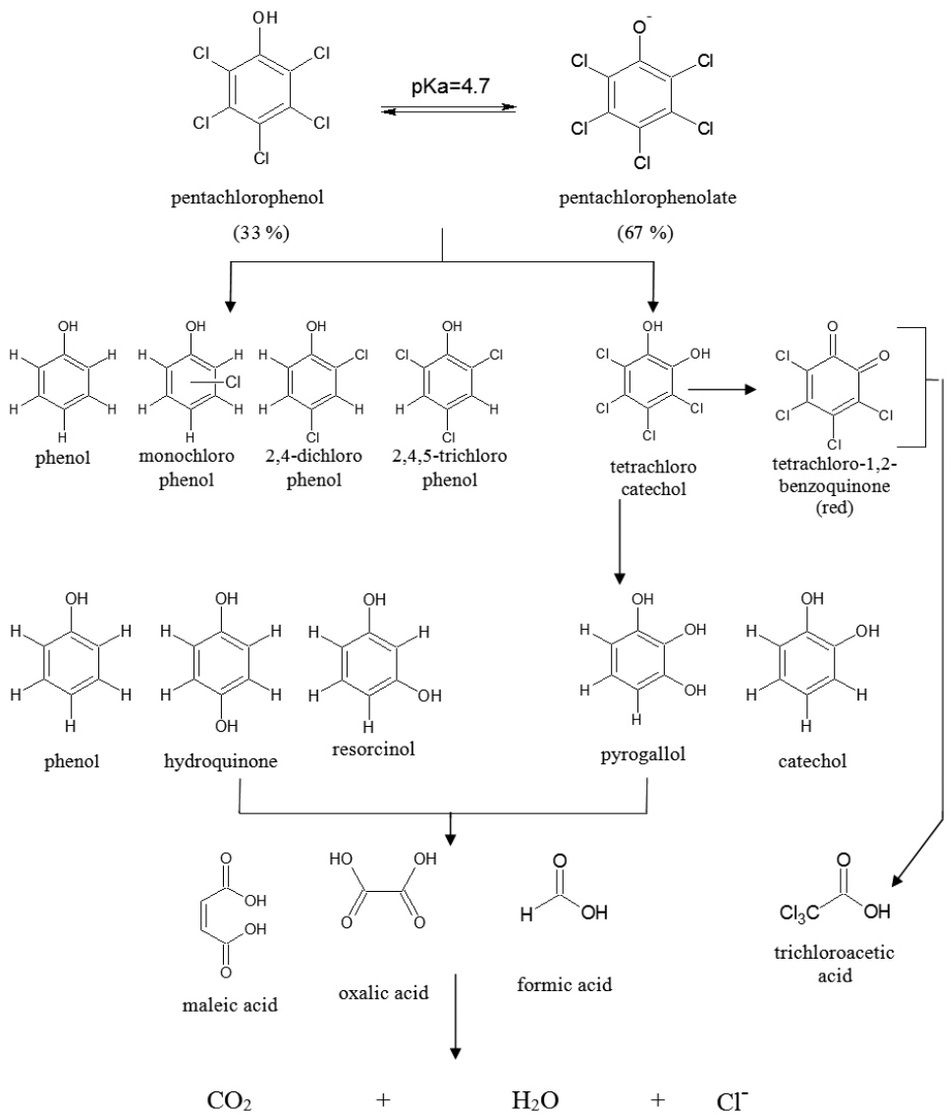


Figure 3: Degradation pathway proposal with the main intermediaries detected during the ozonation process of PCP at pH 5.

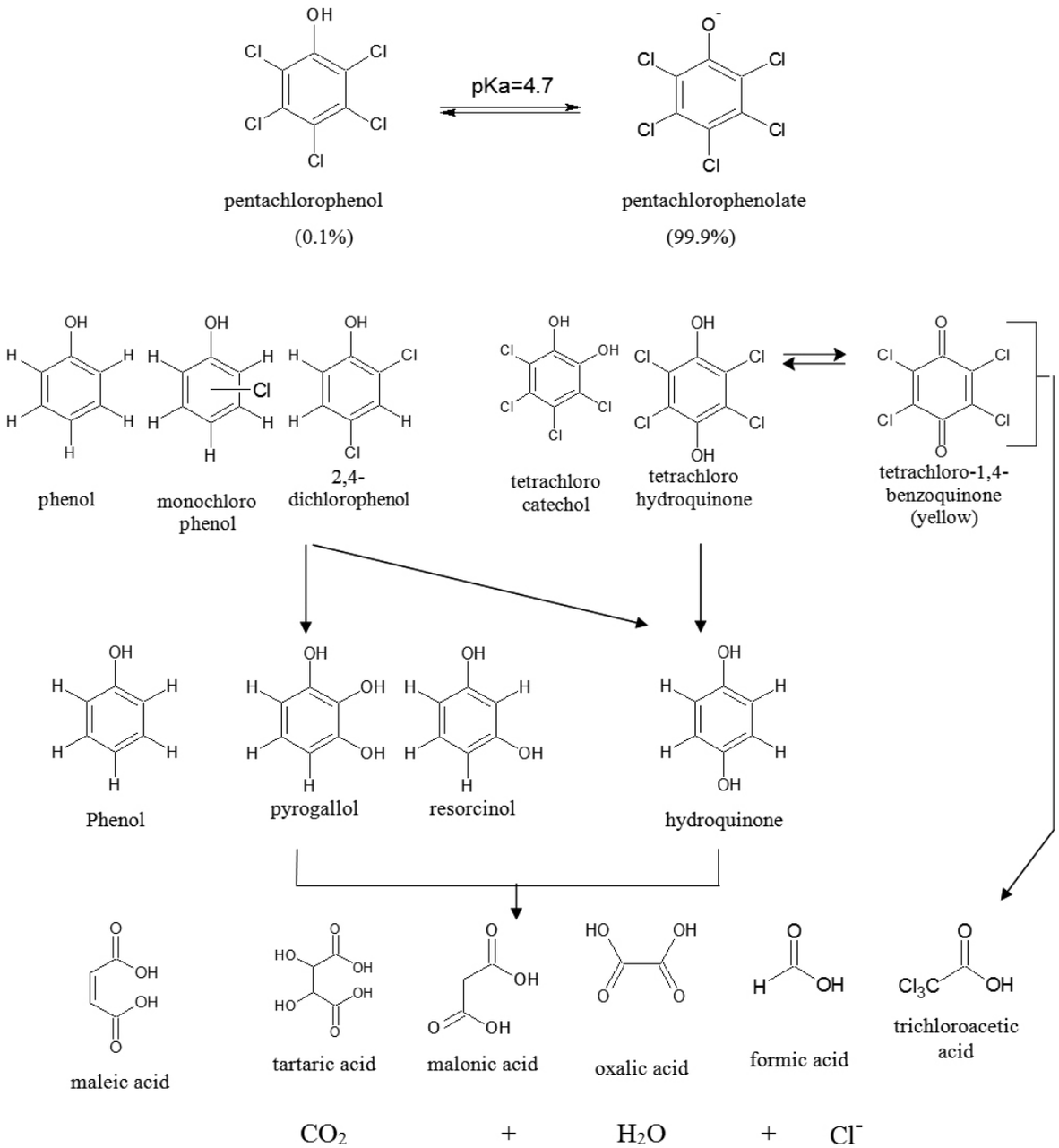


Figure 4: Degradation pathway proposal with the main intermediaries detected during the ozonation process of PCP at pH 9.

3.2 Catalyst Characterization

The preparation of the catalyst MnO_2/TiO_2 was reported previously.[25] **Table 1** summarizes the characterization of the catalysts studied. Some of these results have been previously reported by our group[24].

The textural properties of the catalyst MnO_2/TiO_2 1% were evaluated by nitrogen sorption experiments. The specific surface area and average pore radius obtained from nitrogen adsorption were 43 m^2/g and 5.0 nm, respectively. The specific surface area of the solids obtained from nitrogen adsorption isotherms at 77 K showed the expected trends. The addition of manganese oxide to the support produces a slight decrease in the surface due to surface coverage.

Surface acidity obtained from the TPD of ammonia showed a single, wide peak centered at 533 K for TiO_2. MnO_2/TiO_2 catalyst showed two peaks, the first one was broad and centred at 533 K while the second presented a higher intensity centred at 790 K. **Table 1** presents surface acidity estimated from TPD data. The MnO_2/TiO_2 catalyst displays the highest value. If it is considered that the bulk MnO_2 possess a surface acidity of 0.095 meq g^{-1} and the MnO_2 loading is only 1wt%, only a slight increase should be expected. However, the obtained surface acidity of MnO_2/TiO_2 is approximately 50% higher than the pure support, which is indicative of MnO_2's high surface coverage.

The ZPC values are also displayed in **Table 1**. Taking into account that the bulk TiO_2 displays a ZPC of 6.1, a decrease in 0.5 pH units after the deposition of MnO_2 on TiO_2 is observed. Since the ZPC of bulk MnO_2 is 3.4, an estimate of surface coverage indicates that approximately 20% of the TiO_2 surface is coverage with 1wt% of MnO_2, indicating a high dispersion of the oxide phase.

Table 1. Surface area, surface acidity and ZPC for TiO_2 and TiO_2/MnO_2 catalysts.

Catalyst	S_{BET}, $m^2\ g^{-1}$	Surface acidity, meq g^{-1}	ZPC, pH units
TiO_2	49	0.024	6.1
MnO_2/TiO_2	43	0.037	5.6

The **Fig. 5** shows the XRD (X-Ray Diffraction) of MnO_2/TiO_2 1% w/w. The catalyst has anatase (PDF file 00-021-1272) and rutile (PDF file 00-021-1276) phases, characteristic of titanium oxide; the manganese oxide phase was not be detected, probably due to the low amount of this oxide in the catalyst.

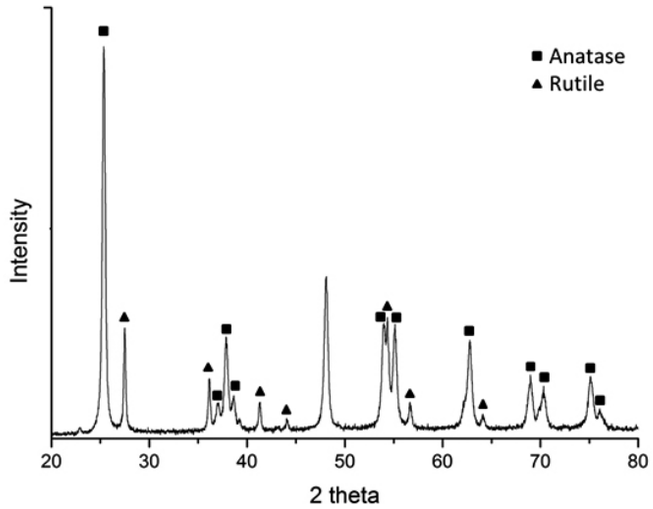


Figure 5: XRD of MnO_2/TiO_2 catalyst.

3.3 Catalytic Ozonation

The catalytic ozonation reactions of PCP were studied at 20ºC and at different pH. At pH 5 the rate constant was $0.7 \pm 0.1\ min^{-1}$, whereas at pH 7 and 9 the k_{obs} was $0.4 \pm 0.1\ min^{-1}$. The slightly higher value of the rate constant at pH 5 can be explained by a major adsorption of phenolate anions on the slightly positive catalyst surface (ZPC = 5.6), allowing a better contact between PCP and oxidant species on the surface of the catalyst.[37]. Nevertheless, adsorption of PCP in the dark is rather small along all the pH values employed. The average amount of PCP adsorbed on TiO_2 was 3.7 % and 4.7 % for TiO_2/MnO_2.

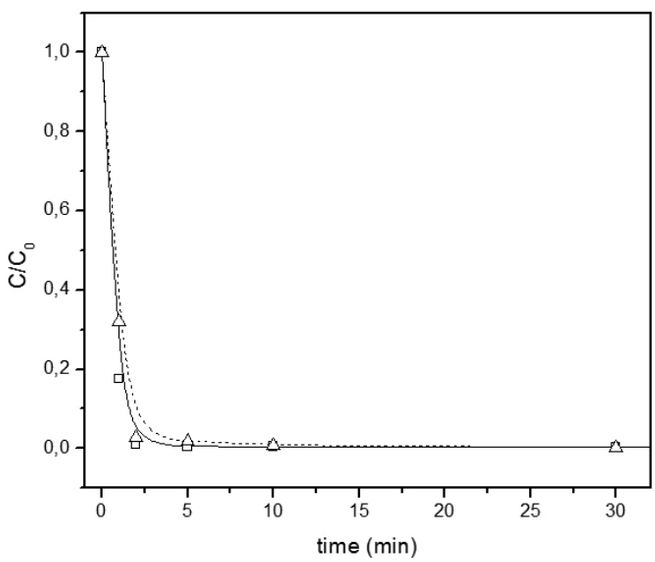


Figure 6: Degradation of PCP at pH 5 during 30 minutes with TiO_2/MnO_2 (□) and without TiO_2/MnO_2 (Δ).

Fig. 6 shows the diminution of PCP concentration over time during the first 30 minutes of reaction at pH 5. Under the experimental conditions employed in this work degradation of PCP is relatively fast, and just a slight difference can be observed.

Although the nature of the intermediates found is the same than in the case of homogeneous ozonation, the effect of the use of TiO_2/MnO_2 is expressed in the change of the proportions of these intermediates. Differences were observed in chloride and CO_2 evolution, which were higher than in a catalyzed presence, indicating a different route to mineralization. Theoretically if the PCP is completely degraded, it would generate $5.6x10^{-4}$ mol/L of chlorides, when comparing the production of chlorides at different pH, it is observed that the highest amount of chlorides formed is at pH9 producing $5.0x10^{-5}$mol/L at the end of the reaction. The greatest degradation of PCP occurs in the first few minutes of the reaction, where the highest CO_2 formation is observed. During ozonation reactions at pH 5 and pH 9, CO_2 was generated reaching concentrations of $7x10^{-6}$mol/L and $2.8x10^{-6}$mol/L respectively, while in the catalytic ozonation reactions, an increase in the CO_2 generation was observed at both pH values, reaching $9.5x10^{-6}$ mol/L at pH 5, and $6x10^{-6}$ mol/L at pH 9 after 40 min of degradation.

3.4 Toxicity assays

One of the objectives of this study was to verify that products generated in the process of degradation of PCP do not show more toxicity than the initial compound. Using *Daphnia magna* as a representative species of an aquatic organism (Ecotoxicity assay) and *Lactuca sativa* and *Panicum millaceum* as terrestrial organism (Phytoxicity assay).

According to several reports, many catalytic processes can generate products that are more toxic than the initial substances. [38,39,40] It was expected that the lower formation of halogenated intermediates would produce less toxic solutions.[41] Our results indicate that the lowest formation of organohalide compounds is performed at pH 9, therefore this system was selected for the following ecotoxicity tests.

3.5 Ecotoxicity assay

The microcrustacean *Daphnia magna* was used according to Chilean Standards. The first assay consisted in evaluating the initial solution of PCP (30 mg/L) at different pH values, because PCP degradation process was dependent on the pH. The results are presented in **Table 3**. It was determined the IC_{50} for PCP with average values of 0.8 mg/L and 0.6 mg/L, at 24 h and 48 h, respectively. An ecotoxicity positive control, IC_{50} for $K_2Cr_2O_7$ (1.2 ± 0.07 mg/L) was included in the assay. These results are reasonably consistent with that previously reported for PCP ($IC_{50\text{-}24h}$ = 0.62 mg/L) using the same microcrustaceans.[42]

Table 3: Results of ecotoxicity with *Daphnia magna* for the model compound PCP.

pH	$IC_{50\text{-}24h}$	$IC_{50\text{-}48h}$
pH 5	0.7 mg/L	0.6 mg/L
pH 7	0.9 mg/L	0.6 mg/L
pH 9	0.8 mg/L	0.6 mg/L

To study the evolution of toxicity during the ozonation reaction, samples from uncatalyzed and catalytic ozonation were taken at different reaction times and diluted to reach approximately twice the $IC_{50\text{-}48h}$. At time zero, when the degradation process had not started, the survival percentage was zero. The percentage of survival increased rapidly when the microcrustacean were incubated with both ozonation samples obtained from 15 minutes of reaction and beyond (data not shown). The decrease in toxicity must be related to the decrease in concentration of PCP and the formation of less toxic intermediates formed during the ozonation process. No significant differences were found in the survival profile between the MnO_2 catalyzed ozonation regarding the uncatalyzed process (data not shown).

3.6 Phytotoxicity assay

Phytotoxicity tests were performed using seeds of *Lactuca sativa* (dicotyledonous) and *Panicum millaceum* (monodicotyledonous). In two tests, the growth of root and stem of both species were measured after 6 days of incubation. The seeds were treated with samples obtained during the degradation of PCP, and distilled water was used as a control. In the case of lettuce assays, it was not observed any significant differences in presence and absence of catalyst.

The **Fig. 7**, shows the growth percentage of root and stem of *Panicum millaceum* exposed to PCP solutions sampled at different time intervals during ozonation and catalytic ozonation at pH9. An initial solution of 30 mg/L PCP (time 0 min) inhibited the root growth ~65% and stem growth ~50%.

It is observed that stem and root growth is favored when the MnO_2/TiO_2 catalyst is used in the degradation process of PCP.

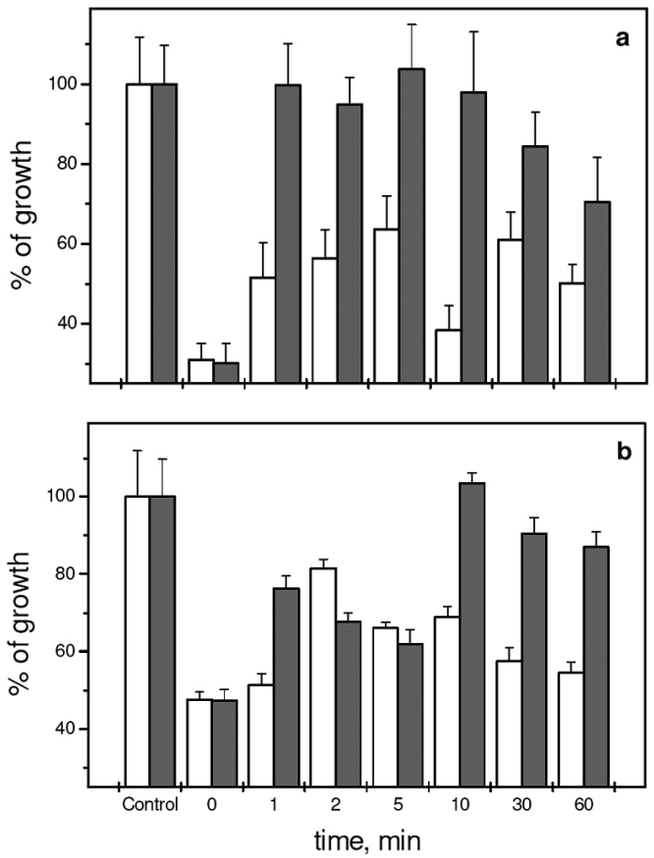


Figure 7. Effect of samples taken at different time intervals during the PCP treatment for ozonation in the presence of (MnO_2/TiO_2) ▓ to pH 9 and in the absence of catalyst, □ expressed as percentage of root growth **(a)** and stem growth **(b)** of germinated seeds of *Panicum millaceum*, compared with a control (water). ($p < 0.05$).

Fig. 7a shows that the root initially grows around 30% observing an increase in growth as the degradation of PCP occurs. In addition, it is observed that when using the catalyst, the growth of the root is very noticeable. This behavior could be explained by the generation of intermediaries in different proportion, **Fig. 7b** where stem growth is observed, also shows that the use of the catalyst helps in the reduction of toxicity, reflected in a greater growth of the stem, observing its greater effect after 10 min of degradation, reaching up to 90% stem growth.

4. CONCLUSION

The ozonation is a dominant process during the degradation of PCP, which achieved ~95% of PCP degradation at 60 min of reaction. It was observed that during the degradation of PCP, quinones were generated as main intermediates that quickly decay leading the formation of several organic acids as degradation products.

The degradation of PCP by ozone was not influenced greatly by pH showing a similar rate reaction in all cases (pH 5, pH 7 and pH 9). This effect was modified when the PCP degradation was done under a catalytic process, producing a higher degradation rate especially under acidic conditions.

Although the nature of the intermediaries produced during degradation of PCP in the presence of MnO_2/TiO_2 catalyst did not show significant differences regarding the degradation products identified in the non-catalytic ozonation processes; a higher production of mineralization products, particularly the amount of chloride generated in the catalyzed ozonation was observed.

The evaluation of ecotoxicity was studied on different biological species to reflect the potential damage that could result in humans. Catalytic ozonation not only allowed >90% of degradation of PCP, but also showed a lower toxicity than the initial. Although a complex mixture of degradation products was produced, they were less toxic than the starting compound.

Regarding the toxicity tests using freshwater species such as *Daphnia magna* and plant species such as *Lactuca sativa* and *Panicum millaceum*, they were highly affected by the untreated PCP solution (30 mg/L), while the same species treated by simple ozonation and catalytic ozonation showed a better response demonstrating the efficiency of the toxicity reduction. There is a close relationship between toxicity and dechlorination of PCP. Accordingly, it was observed that the MnO_2/TiO_2 catalyst lowered the toxicity of the treated solution, thereby permitting a better growth of root and stem in the phytotoxicity tests.

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