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Sequential UV–biological degradation of chlorophenols

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Abstract

The sequential UV–biological degradation of a mixture of 4-chlorophenol (CP), 2,4-dichlorophenol (DCP), 2,4,6-trichlorophenol (TCP), and pentachlorophenol (PCP) was first tested with each pollutant supplied at an initial concentration of 50 mg l^{-1}. Under these conditions, the chlorophenols were photodegraded in the following order of removal rate: PCP > TCP > DCP > CP with only CP and DCP remaining after 40 h of irradiation. The remaining CP (41 mg l^{-1}) and DCP (13 mg l^{-1}) were then completely removed by biological treatment with an activated sludge mixed culture. Biodegradation did not occur in similar tests conducted with a non-irradiated mixture due to the high microbial toxicity of the solution. UV treatment lead to a significant reduction of the phytotoxicity to *Lipedium sativum* but no further reduction of phytotoxicity was observed after biological treatment. Evidence was found that the pollutants were partially photodegraded into toxic and non-biodegradable products. When the pollutants were tested individually (initial concentration of 50 mg l^{-1}), PCP, TCP, DCP, 4-CP were photodegraded according to first order kinetic model ($r^2 > 99$) with half-lives of 2.2, 3.3, 5.7, and 54 h, respectively. The photoproducts were subsequently biodegraded. This study illustrates the potential of UV as pre-treatment for biological treatment in order to remove toxicity and enhance the biodegradability of organic contaminants. However, it also shows that UV treatment must be carefully optimized to avoid the formation of toxic and/or recalcitrant photoproducts and results from studies conducted on single contaminants cannot be extrapolated to mixtures.
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1. Introduction

Chlorophenols have been widely used as bactericides, insecticides, herbicides, fungicides and wood preservative as well as intermediates of dyes (Czaplicka, 2004). They are released in the environment from direct use, accidental spillage, or degradation of other substances. Therefore, they are commonly detected in soil, sediments, surface water, and wastewater and cause severe environmental problems (Czaplicka, 2004; Pera-Titus et al., 2004). Unfortunately, chlorophenols are highly toxic and tend to persist in the environment, which is why they have been listed as priority pollutants by the US EPA and the EU (Pera-Titus et al., 2004). Hence, suitable methods must be developed to remediate contaminated sites.

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Biological remediation methods are often cheaper and more environmentally friendly than their physical or chemical counterparts (i.e., incineration, ozonation). Unfortunately, they are also less versatile as microbial activity is more easily affected by process parameters such as the effluent toxicity. Hence, a more cost-efficient alternative consists in combining physical and chemical processes with biological methods (Scott and Ollis, 1995; Marco et al., 1997; Mantzavinos and Psillakis, 2004). More precisely, chemical oxidation can serve to break down toxic and/or poorly biodegradable pollutants into less toxic and/or more biodegradable molecules that can then be easily biodegraded (Zeng et al., 2000; Sarria et al., 2001, 2002; Amat et al., 2003; Goi et al., 2004). This saves a considerable amount of energy in comparison with what is needed to achieve the full mineralization of the pollutants by chemical oxidation (Marco et al., 1997). The challenge is to optimize the two processes simultaneously and make sure that the degradation products are biodegradable and do not inhibit the subsequent biological stage.

With this perspective, the physical–biological treatment of 4-chlorophenol (4-CP), 2,4-dichlorophenol (DCP), 2,4,6-trichlorophenol (TCP), and pentachlorophenol (PCP) was evaluated. UV-irradiation was selected as the pre-treatment technology because of its effectiveness towards aromatic pollutants (Guieysse et al., 2004; Guieysse and Viklund, 2005). UV-irradiation can also be based on solar energy to lower treatment costs (Malato et al., 2002).

2. Materials and methods

All chemicals were reagent grade. 4-CP, DCP, TCP, and PCP were obtained from Sigma-Aldrich. Stock solutions of DCP ($5\ g\,l^{-1}$), TCP ($1\ g\,l^{-1}$), PCP ($1\ g\,l^{-1}$), and 4-CP ($5\ g\,l^{-1}$) were prepared in pure water (Millipore purification system) alkalified with 0.1% 2 N NaOH. All experiments were conducted in triplicate at room temperature ($23 \pm 2\ ^{\circ}C$). In the following, even when the pollutants are tested as mixtures, the chlorophenol concentrations given are the concentrations of each compound in the mixture (e.g., not the total pollutant concentration).

2.1. UV treatment

UV-irradiation tests were performed into 10 ml quartz tubes filled with 6 ml of Mineral Salt Medium (MSM) supplied with CP, DCP, TCP, and/or PCP. The MSM was composed of ($mg\,l^{-1}$ of deinonized water): K_2HPO_4 4000, Na_2HPO_4 5200, KNO_3 3000, $CaCl_2 \cdot 7H_2O$ 10, $MgSO_4 \cdot 7H_2O$ 500, $FeSO_4 \cdot 7H_2O$ 10, $MnCl_2 \cdot 4H_2O$ 5.5, $ZnCl_2$ 0.68, $CoCl_2 \cdot 6H_2O$ 1.2, $NiCl_2 \cdot 6H_2O$ 1.2, $CuCl_2 \cdot 2H_2O$ 0.85, H_3BO_3 0.0031, $NaMoO_4 \cdot 2H_2O$ 0.012, $NaSeO_3 \cdot 5H_2O$ 0.013, and $NaWO_4 \cdot 2H_2O$ 0.0165. The pH was adjusted to 7 with 2 N NaOH. This medium was used for being suitable for the subsequent biodegradation studies (Guieysse et al., 2004; Guieysse and Viklund, 2005). Nitrate was preferred to ammonium as nitrogen source to avoid microbial growth as a result of nitrification (since biodegradation tests were conducted under aerobic conditions for 28 d). The phosphate buffer was used to prevent from pH variation during UV-irradiation and biodegradation tests (the pH value was controlled after UV irradiation and biodegradation tests and always remained to ≈7).

The tubes were mechanically agitated using a rocking shaker and irradiated at 300 $\mu W/cm^2$ (at 15 cm) for 40 h with two 18 W UV blue-lamps (Sylvania Reptistar, Sylvania, USA, ≈30% UVA–5% UVB) placed 15 cm away from the tubes. Samples of 100 and 500 μl were periodically withdrawn for measuring the chlorophenol and chloride concentrations, respectively. They were saved at 4 °C when necessary. For the COD and phytotoxicity analysis, samples of 10.0 and 8.0 ml, respectively, were withdrawn at the beginning and end of UV treatment and immediately treated.

2.2. Biodegradation tests

After irradiation, aliquots of 6.0 ml were transferred from the quartz tubes in 12 ml test tubes and inoculated with 400 μl of an activated sludge sample (Lund wastewater treatment plant, Lund, Sweden). The inoculum furnished the typical microflora found in wastewater treatment and contained a high microbial diversity, which should favor the mineralization of chlorophenol photoproducts. For each pollutant or mixture of pollutants tested, control tests were prepared using non-irradiated solutions. In addition, to estimate the potential abiotic removal, non-inoculated control tubes were prepared. In order to allow for microbial acclimation, all tubes were incubated in the dark on a rotary shaker (150 rpm) for 28 d, as suggested by the Zahn–Wellens/EVPA biodegradability test (OECD, 1993). Samples were periodically withdrawn for chloride (500 μl) and chlorophenol (100 μl) analysis. The COD and phytotoxicity were measured again at the end of the incubation. Each experiment was done in triplicates.

For monitoring microbial activity, the same procedure was repeated by using aliquots of 6.0 ml in 20-ml serum flask inoculated with 400 μl of each inoculum using the same set of controls. The flasks were closed with rubber septa and aluminum caps and incubated in the dark on a rotary shaker (150 rpm) for 28 d. Samples were periodically withdrawn from the flasks' headspaces for the monitoring of the gas phase composition.

2.3. Characterization of photoproducts

For each chlorophenol, a 500 mg l^{-1} solution in MSM was irradiated for 56 h. Samples were withdrawn at time zero and after 56 h for analysis by LC/MS.

2.4. Analysis

The UV-absorption spectra of DCP, TCP, and PCP at 100 mg l^{-1} in water were measured to determine the maximum excitation coefficient of each compound between 200 and 700 nm. The UV-spectra were determined with a V/Visible Spectrophotometer Ultrospec 1000 (Biochrom, England).

HPLC analyses were performed using a Lachrom L-7000 liquid chromatograph with a Lachrom L-7250 autosampler and L-7400 variable wavelength monitor. CP, DCP and TCP were eluted through a LC-8 column (Supelco) with a mobile phase composed of methanol:water:acetic acid (60:39:1 v/v). The UV detection was performed at 280 nm. PCP was eluted through a LC-18 column (Supelco) with a mobile phase composed of acetonitrile:water (80:20 v/v). Here the UV detection was conducted at 302 nm. External standards were used to enable quantitative determination and the limit of detection was 1 mg l^{-1} for all compounds. Samples were centrifuged for 10 min at 13 000 rpm (Biofuge 13, Heraeus) and a portion of the supernatant was transferred into HPLC vials prior to analysis.

Samples for LC–MS were analyzed using a LC-8 column as described above or using the LC-18 column eluted with an acetonitrile:water (50:50 v/v) mobile phase.

COD analyses were performed using LCK 414 test tubes (Dr Lange) and a Lasa 100 photometer (Dr Lange) equipped with a LT 100 heater (Dr Lange). Samples were centrifuged for 10 min at 13 000 rpm (Biofuge 13, Heraeus) and a portion of the supernatant was transferred into the test tubes for analysis.

Sample for chloride analysis were centrifuged for 10 min at 13 000 rpm (Biofuge 13, Heraeus) and portion of the supernatant was transferred into test tubes. Chloride concentration was measured by flow injection analysis using a FIA star 5000 analyzer (Foss) according to the manufacturer's instructions. The absorbance of the solution was measured at 470 nm.

To measure the microbial respiration rate, gas samples of 100 μl were withdrawn from the closed bottles using a syringe and injected on a GC (Varian 3350) equipped with a thermal conductivity detector. The carrier gas was helium at a flow rate of 12 ml min^{-1} and the column used was a Haysep Q, 80–100 mesh, 2 m × 1/8∅ × 2 mm. The column, injector and detector temperatures were 70, 90, and 150 °C, respectively.

Phytotoxicity tests were conducted in glass dishes. Five seeds of *Lipedium sativum* were placed on a 5.5 cm (∅) filter paper and contacted with 2.0 ml of sample (pH 7). The dishes were then covered and incubated in complete darkness for 5 d. When necessary, the samples were diluted 10 times and/or centrifuged for 10 min at 4000 rpm (Mistral 1000) before measuring the toxicity. Controls were done with tap water without pollutants. Three Petri dishes were prepared for each test and the toxicity effect was calculated as a ratio (in %) of the average stem length of the 15 test seeds and the average stem length of the 15 control seeds. Potential outliers were identified and rejected by using the Grubb's test at the 5% significant level.

All of the results represent the average from 15 (phytotoxicity) or 3 (other analysis) replicates ± the standard deviation of these replicates. When necessary, the results were analyzed with one way ANOVA (p-value $\leqslant 0.05$).

3. Results

3.1. UV–biological treatment of chlorophenols in mixture (100 mg l^{-1})

After 56 h of irradiation, CP, DCP, TCP remained to approximately 86 ± 1%, 42 ± 7%, and 9 ± 4% of their initial concentrations, respectively. PCP was completely removed. Inoculation of the irradiated mixture was not followed by pollutant removal or microbial activity (O_2 consumption or CO_2 release) during the 28 d of incubation. Microbial growth was recorded in the 3-fold diluted irradiated mixture after re-inoculation and the complete removal of the remaining 4-CP and DCP was achieved after 3 d of incubation. TCP remained at 5 ± 0 mg l^{-1} after 13 d of incubation. Neither pollutant removal nor microbial activity were recorded in the inoculated non-irradiated mixture used as a control.

3.2. UV–biological treatment of chlorophenols in mixture (50 mg l^{-1})

UV irradiation was followed by the disappearance of the chlorophenols and the release of chloride (Fig. 1). The photodegradation of PCP, TCP, and DCP was well described by a first order kinetic with half lives of 3.5, 5.9, and 21.5 h, respectively ($r^2 > 0.94$). CP was only removed by 15%. The COD decreased from 220 to 176 mg l^{-1} at the end of irradiation and the concentration of chloride increased from 0 to 49.7 mg l^{-1} (Table 1). *L. sativum* stem growth was completely inhibited by the non-irradiated mixture. After the UV treatment, no inhibition effect was reported in the 10-fold diluted sample, though the stem growth was still inhibited by ≈90% in the undiluted sample.

Biological treatment of the irradiated mixture was followed by the disappearance of the remaining DCP and CP after 5 and 7 d of incubation, respectively. It

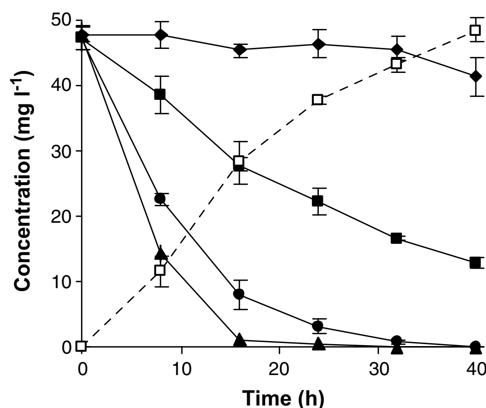


Fig. 1. Change of CP (diamonds), DCP (squares), TCP (circles), PCP (triangles) and chloride (open square) concentrations in quartz tubes supplied with a mixture of the 4 chlorophenols ($50\ mg\ l^{-1}$) in MSM and submitted to UV irradiation for 40 h.

was also followed by a reduction of the COD but there was no significant increase of the dechlorination efficiency or decrease of phytotoxicity (Table 1). CO_2 production and O_2 consumption were also only recorded in the inoculated flasks supplied with the irradiated mixture and stabilized after ≈7 d of incubation (data not shown).

3.3. *UV–biological treatment of chlorophenols supplied individually ($50\ mg\ l^{-1}$)*

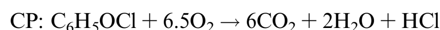
The photodegradation of the chlorophenols fitted a first order kinetic model ($r^2 > 99$) with half-lives of 2.2, 3.3, 5.7, and 54 h for PCP, TCP, DCP, 4-CP, respectively (Fig. 2). For all contaminants, chloride release occurred but was delayed in comparison to pollutant removal (Fig. 2). After inoculation, the remaining 4-CP was removed in 5 d and chloride was released for all contaminants (Fig. 3). In total, the combined UV–biological treatment allowed for the complete removal of all contaminants with dechlorination efficiencies higher than 95% and COD removal of 76–86% (Table 2).

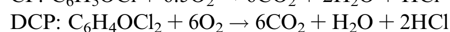
In the inoculated tests supplied with non-irradiated solutions, complete removal of 4-CP and DCP occurred within 7 and 8 d, respectively (Fig. 4). Complete removal of TCP took 20 d due to a 12 d long lag phase. No significant removal of PCP was observed during the 28 d incubation period. Except for PCP, chloride was released in all the inoculated tubes and at the same time as the pollutant was removed (Fig. 4).

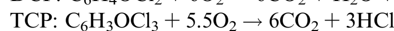
Table 1
Effect of UV–biological treatment of mixture of CP, DCP, TCP, and DCP supplied at $50\ mg\ l^{-1}$ each

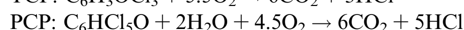
Treatment		No[a]	UV[b]	Biol.[c]	UV + Biol.[d]
Phytotoxicity (%)	No dilution	100	89 ± 11	100	75 ± 4
	10 times dilution	35 ± 17	0	24 ± 14	0
COD ($mg\ l^{-1}$)		220 ± 8[f]	176 ± 5	214 ± 12	123 ± 8
COD removal (%)		0	20 ± 1	3 ± 0	44 ± 3
Cl ($mg\ l^{-1}$)		0	52 ± 1	0	67 ± 0
Dechlorination[e] (%)		0	68 ± 4	0	72 ± 3
Pollutant removal (%)	CP	2 ± 0	15 ± 0	9 ± 0	100
	DCP	7 ± 0	78 ± 0	9 ± 1	100
	TCP	0	100	0	100
	PCP	9 ± 1	100	13 ± 1	100

[a] Abiotic control supplied with a non-irradiated mixture and incubated in the dark for 28 d.
[b] Abiotic control supplied with an irradiated mixture and incubated in the dark for 28 d.
[c] The mixture of chlorophenols was not irradiated but inoculated and incubated in the dark for 28 d.
[d] The mixture of chlorophenols was irradiated for 40 h then inoculated and incubated in the dark for 28 d.
[e] Dechlorination (%) = $[Cl]/[Cl_{th}]$, where [Cl] is the concentration of free chloride measured in solution after treatment and $[Cl_{th}]$ is the concentration of chloride theoretically released from the amount of contaminant removed. $[Cl_{th}]$ was derived from the following oxidation reaction:


CP: $C_6H_5OCl + 6.5O_2 \rightarrow 6CO_2 + 2H_2O + HCl$


DCP: $C_6H_4OCl_2 + 6O_2 \rightarrow 6CO_2 + H_2O + 2HCl$


TCP: $C_6H_3OCl_3 + 5.5O_2 \rightarrow 6CO_2 + 3HCl$


PCP: $C_6HCl_5O + 2H_2O + 4.5O_2 \rightarrow 6CO_2 + 5HCl$

Thus: $[Cl_{th}] = 0.276\ [CP] + 0.436\ [DCP] + 0.539\ [TCP] + 0.533\ [TCP]$ (concentration given in $mg\ l^{-1}$).

[f] The theoretical COD for each contaminant was calculated according to the same equations: $COD_{CP} = 1.62\ g\ g^{-1}$; $COD_{DCP} = 1.18\ g\ g^{-1}$; $COD_{TCP} = 0.89\ g\ g^{-1}$; and $COD_{PCP} = 0.54\ g\ g^{-1}$. Hence, for the original mixture at $50\ mg\ l^{-1}$, the COD was $211.4\ g\ g^{-1}$.

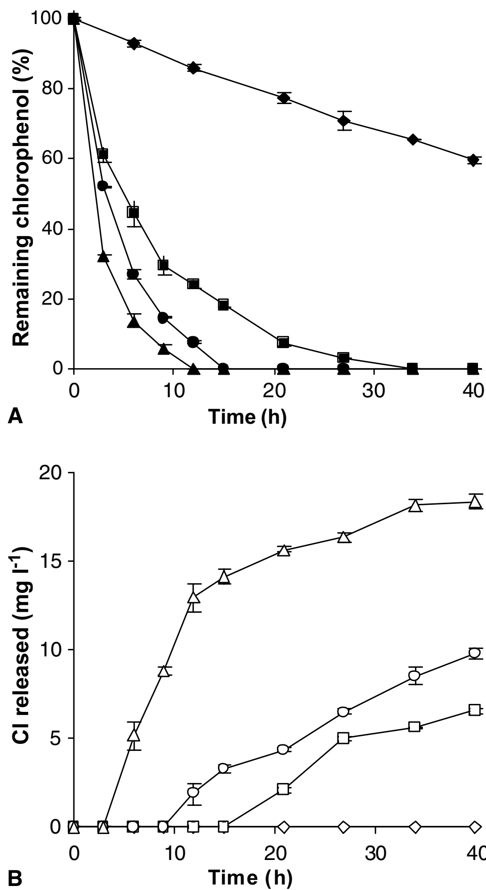


Fig. 2. Change in pollutant remaining level (A) and chloride concentration (B) in quartz tubes supplied with 50 mg l^{-1} of CP (diamonds), DCP (squares), TCP (circles) or PCP (triangles) in MSM and submitted to UV irradiation for 40 h.

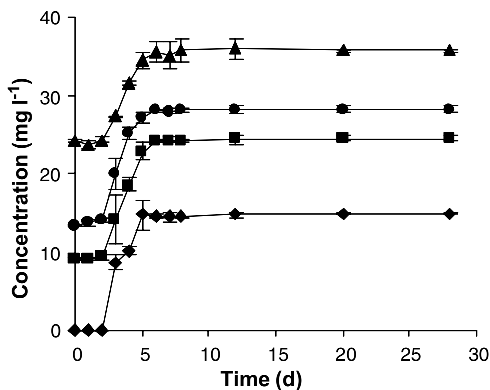


Fig. 3. Change in chloride concentration in cultivation tubes supplied with UV-irradiated (40 h) solutions initially containing 50 mg l^{-1} of CP (diamonds), DCP (squares), TCP (circles) or PCP (triangles) in MSM, inoculated with an activated sludge sample and incubated for 28 d.

Before irradiation, all contaminants completely inhibited the germination of *L. sativum* at 50 mg l^{-1} (Table 2). After irradiation, the phytotoxicity in undiluted samples remained very high in all samples (≈100%), but the 10-fold diluted solutions of irradiated PCP or TCP were no longer toxic (Table 2). For CP, DCP, and TCP, the reduction of phytotoxicity was in the same order of magnitude after biological treatment, regardless if the samples were irradiated or not. For PCP, there was no recorded reduction of phytotoxicity in the undiluted solution after UV or biological treatment alone. However, the phytotoxicity decreased to ≈58% after the combined UV–biological treatment.

3.4. Characterization of photoproducts

There was no clear pattern of photoproduct formation as many peaks appeared and disappeared during irradiation. From the analysis using the LC-8 column, the largest peaks were identified as chatechol or hydroquinone. From the analysis using LC-18 column, detected peaks were identified as chatechol, mono chlorohydroquinone, trihydroxy chlorobenzene, dichloro *p*-benzoquinone, and trihydroxy trichlorobenzene. With the LC18 column and TCP sample, a peak was identified as 3,5 dichlorocatechol.

4. Discussion

The UV-irradiation of a mixture of PCP, TCP, DCP, and CP initially supplied at 100 mg l^{-1} was followed by a significant removal of PCP, TCP, and DCP. However, no microbial activity was recorded in the irradiated mixture after inoculation. Instead, microbial activity and pollutant removal were only recorded after re-inoculation of the same solution 3-fold diluted. This showed that the microbial toxicity of the non-diluted irradiated mixture was too high to allow microbial growth.

When the same experiment was repeated at initial concentrations of 50 mg l^{-1}, the CP and DCP remaining in the irradiated mixture were completely biodegraded. No significant changes in microbial activity or chloride release were recorded in the non-irradiated inoculated mixture (Table 1), suggesting that the combined toxicity of the pollutants in the non-irradiated mixture inhibited microbial growth. The concentrations of CP, DCP, and PCP decreased by 9–13%, but was likely caused by pollutant absorption to the due since pollutant removal in the same order of magnitude was recorded in the abiotic control. Hence, UV-irradiation was a suitable pre-treatment to reduce the toxicity of the pollutant mixture, thereby facilitating microbial growth and permitting the biological degradation of the remaining pollutants.

The combined UV–biological treatment allowed the complete removal of all contaminants, but only a

Table 2
Effect of UV irradiation and biological treatment on CP, DCP, TCP, and PCP provided alone at 50 mg l^{-1}

Treatment		CP	DCP	TCP	PCP
Initial					
Phytotoxicity (%)	No dilution	96 ± 6	96 ± 6	100	100
	10-fold dilution	0	0	8 ± 5	50 ± 3
COD (mg l^{-1})		84 ± 4	64 ± 1	49 ± 1	31 ± 0
UV treatment					
Phytotoxicity (%)	No dilution	98 ± 4	96 ± 6	100	98 ± 3
	10-fold dilution	0	0	0	0
COD removal (%)		15 ± 1	37 ± 1	33 ± 1	23 ± 1
Pollutant removal (%)		41 ± 1	100	100	100
Dechlorination (%)		0	31 ± 1	36 ± 1	48 ± 2
Biological treatment					
Phytotoxicity (%)	No dilution	55 ± 5	55 ± 4	58 ± 5	100
	10-fold dilution	0	0	0	54 ± 6
COD removal (%)		85 ± 4	82 ± 4	78 ± 1	0
Pollutant removal (%)		100	100	100	6 ± 0
Dechlorination (%)		97 ± 4	100 ± 2	94 ± 1	0
UV + Biological treatment					
Phytotoxicity (%)	No dilution	54 ± 4	52 ± 4	60 ± 6	58 ± 5
10-fold dilution		0	0	0	0
COD removal (%)		84 ± 4	82 ± 3	81 ± 1	76 ± 0
Pollutant removal (%)		100	100	100	100
Dechlorination (%)		96 ± 3	101 ± 2	95 ± 2	96 ± 1

COD removal of 44% and a dechlorination efficiency of 72%. This suggested that some photoproducts released from the UV irradiation remained recalcitrant to biological attack. The COD removal observed after UV treatment was largely due to the biodegradation of the remaining CP and DCP (calculation not shown). This also explains why the dechlorination efficiency (calculated from the amount of pollutant actually removed) and the detoxification efficiency did not significantly increase after biological treatment of the irradiated mixture (Table 1). Common photoproducts of chlorophenols are chlorinated chatechols, polychlorinated hydroxybenzenes, polychlorinated hydroquinones, hydroquinones, and some oxalic and formic acids (Miller et al., 1988; Durant and Brown, 1995; Skurlatov et al., 1997; Benitez et al., 2003). These are normally considered as rather biodegradable (Scott and Ollis, 1995). However, Svenson and Hynning (1997) recorded an increase in toxicity after photolytic treatment of TCP because of the formation of 3,5-dichlorochatechol, which was more toxic than TCP. Likewise, Grabner et al. (1994) reported the formation of polychlorinated biphenyls as products of 4-CP photolysis, showing that the photolysis of chlorophenols may lead to formation of more recalcitrant molecules.

Therefore, a new series of experiment was conducted with the pollutant tested individually at 50 mg l^{-1} to better understand the UV degradation pathway and its consequence during subsequent biological treatment. This time, the photoproducts released from PCP, TCP, and DCP were clearly biodegraded because the COD removal efficiencies and dechlorination activities increased after biological treatment of the irradiated solutions where the chlorophenols had been completely photodegraded. There was no clear pattern of metabolite formation from the UV pre-treatment mixture of pollutants. It is therefore unclear why only the photoproducts released from the UV pretreatment of the mixture did not biodegrade further. An interesting observation was that dechlorination started after pollutant removal during UV-irradiation (Fig. 2) whereas the two phenomena occurred simultaneously during biological treatment (Fig. 4). Perhaps the initial mechanism of UV-irradiation did not start by the removal of a chlorine atom but this has never been reported in the literature, to the best of our knowledge. Nevertheless, the mechanisms of photobiodegradation were different within the mixture of contaminants than when irradiating pure compounds, and recombination of photoproducts might have caused the formation of recalcitrant molecules in the mixture.

Under all experimental conditions tested, the pollutants were photodegraded in the following order: PCP > TCP > DCP > CP, which was the same order than the excitation coefficient of these compounds at 320 nm (data not shown). This is similar to the observations of Shen et al. (1995) who reported faster TCP photodegradation than DCP and CP when irradiating these

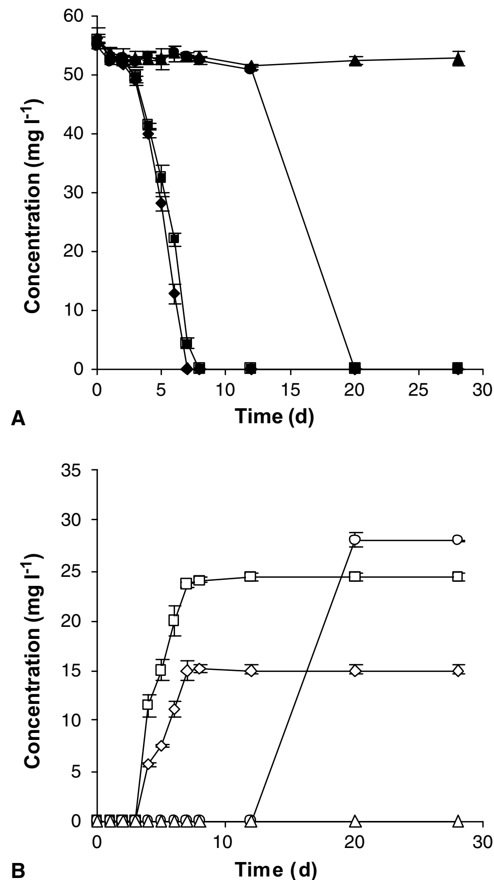


Fig. 4. Change in pollutant concentration (A) and chloride concentration (B) in cultivation tubes supplied with 50 mg l^{-1} of CP (diamonds), DCP (squares), TCP (circles) or PCP (triangles) in MSM, inoculated with an activated sludge sample and incubated for 28 d.

compounds in aqueous solution (pH 7) using a 254 nm low pressure UV lamp, and of Miller et al. (1988) who reported faster TCP photodegradation than DCP when irradiating the samples in water using a UV lamp emitting between 270 and 300 nm. Except for CP, the photodegradation of all pollutants was always well described by a first order kinetic model, as previously reported (Benitez et al., 2000a,b; Pandiyan et al., 2002). The photodegradation rates reported here were in the range of Hwang et al. (1986) who reported half-lives of 0.6–3 h for DCP, TCP, and PCP and 118 h for CP when sunlight was used as source of radiation.

According to the phytotoxicity tests conducted in this study, the chlorophenols tested can be ranked according the following order of toxicity: PCP > TCP > DCP = CP. This is in the same order as the 50% inhibition values reported for these compounds for *Photobacterium phosphoreum* (the Dictionary of Substances and their Effects). The biodegradability of the chlorophenols can also be ranked according the following molar volumetric removal rates (mM d^{-1}) as CP (0.1) ≅ DCP (0.1) > TCP (0.03) > PCP (0). This corroborates with the common assumption that the biodegradability of chlorophenols decreases with the number of chlorine atoms (Annachhatre and Gheewala, 1996). Hence, the UV and biological removal processes complimented well each other as UV irradiation preferentially targeted the least biodegradable and most toxic pollutants (TCP and PCP).

For CP, DCP, and TCP, biological treatment was more efficient in terms of phytotoxicity removal, COD removal, and dechlorination than UV treatment alone. However, UV pre-treatment was necessary to reduce the microbial toxicity of the contaminant mixture and for PCP since it allowed for the formation of photoproducts that were completely dechlorinated and at least partially mineralized (the remaining COD levels were very low and could be attributed to compounds found in the MSM or to biological products). Complete phytotoxicity removal was never observed but toxicity levels of ≈50% were recorded in filtrated domestic wastewater samples, showing that a 50% inhibition level can be regarded as satisfactory for this particular case.

5. Conclusions

The UV treatment of a mixture of CP, DCP, TCP, and PCP (50 mg l^{-1} each) allowed for the complete removal of PCP and TCP, and partial removal of DCP and CP. This lead to a significant reduction of plant and microbial toxicity. It was therefore possible to biodegrade the remaining CP and DCP, though evidence was found that some photoproducts released during UV irradiation were not biodegradable and exhibited a significant phytotoxicity. Hence, UV treatment alone is not satisfactory even when complete pollutant removal is observed. This study also illustrates that results from single pollutants cannot be extrapolated to predict the fate of contaminants within mixtures. Further work should focus on understanding the pathways of photoproduct formation within contaminant mixtures and pre-treating the pollutants using advanced oxidation processes (Sarria et al., 2002).

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