VARIATION OF TOXICITY DURING THE OZONATION OF MONOCHLOROPHENOLIC SOLUTIONS

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ABSTRACT

This study investigates the variation of toxicity during ozonation of 2-chlorophenol (2-CP), 3-chlorophenol (3-CP) and 4-chlorophenol (4-CP) in neutral condition. Acute toxicity of pure chlorophenols (CPs) and their ozonated intermediates was evaluated by Microtox assay. The results revealed that the intermediates of oxidized CPs induced new toxicity during the early stage of ozonation, and the ozonated 2-CP showed higher degree of toxicity increase than 3-CP and 4-CP. The maximum toxicity normally occurred before the maximum color intensity was monitored, while ozone dosage applied was within 1 mg of ozone per mg of initial CPs. This increasing toxicity was mainly contributed from ozonated intermediates. Formation of chlorocatechols, chloromuconic acids and hydroxylated/chlorinated dimeric compounds were detected in ozonation of CPs. These chlorinated by-products may cause greater toxicity than the parent chlorophenols. The required ozone dosage to detoxify the CPs solution into a complete non-toxic condition follows the order: 4-CP > 3-CP > 2-CP.

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INTRODUCTION

Many aromatic compounds, including the chloro-, nitro-substituted aromatic compounds or substituted phenols have been reported to be quite bioresistant in the nature and in treatment plants. One of these compounds, the chlorophenols (CPs) are typically biorefractory or toxic in the aqueous environment due to their chloro substituent (1). Related studies have examined the feasibility of using partial oxidation of chlorophenols by ozone or other oxidants (Advanced Oxidation Processes, AOPs) to promote biodegradability for subsequent biological treatment and to reduce the toxicity of effluent (2–5). In general, the byproducts formed in these oxidation processes are mainly low molecular weight (organic acids) or hydroxylated compounds, which are presumed to be more biodegradable or less toxic than their parent compounds (6,7). Therefore, Adams et al. (8) indicated that the ozonation of 4-CP and 2,4-dichlorophenol (DCP) significantly enhanced their biodegradability by the unacclimated microbial seed obtained from a domestic wastewater treatment plant's activated sludge, and that the ultimate COD (Chemical Oxygen Demand) removal increased about 50% for these two chlorophenols. Trapido et al. (2) also reported that ozonation of CPs (2-CP, 4-CP, 4-chlororesorcinol, 6-chloro-*m*-cresol and 2,4-dichlorophenols) decreases their toxicity according to the *Daphnia magna* 24 h toxicity test when ozone treatment lead to 90–95% removal of CPs.

On the other hand, the toxic intermediates of oxidative treatment prior to the detoxification of the chlorinated aromatic compounds have been reported (9–11). Hirvonen et al. (9) reported that the hydroxylation of chlorophenols and formation of dimeric intermediates were involved in the oxidation of chlorophenols by ozone and UV/H_2O_2 processes. Hydroxylated chlorophenols, chlorodihydroxybiphenyls and dichlorodihydroxybiphenyls were formed in both oxidation treatments of monochlorophenols (2-CP and 4-CP). These byproducts showed the characteristics of high molecular weight or the presence of chlorinated intermediates, which present toxic concern. Svenson and Hynning (10) indicated that photolytic degradation of 2,4,6-trichlorophenol was found to increase aqueous toxicity measured by the Microtox bioassay system, and this increasing toxicity was mainly generated from 3,5-dichlorocatechol formed. In light of above development, oxidation treatment of mono-chlorophenols by ozone is studied in this investigation, and attention is focused on the variation of toxicity. Acute toxicity of pure CPs and their intermediates are evaluated by the Microtox toxicity assay (12).

MATERIALS AND METHODS

Process of Ozonation

Figure 1 shows a schematic diagram of the ozonation apparatus employed as a semi-batch type. Ozonation is performed in a lab-scale bubble column reactor (327 mm high and 70 mm diameter) with a magnetic stirrer. All the components in this system are made from acrypoly glass, Teflon or stainless steel. The ozone generator used was manufactured by JEU TU CO., Japan (Series No, SG-01A-PSA4). The ozone gas was introduced into a diffuser in the bottom of reactor and the concentration in the feed-gas was maintained at 77.0 mg/l ($[O_3]_{in}$). The gas flow rate was 976 ml/min (q) in all experiments. The concentration of ozone gas was measured by the potassium iodide technique based on the Standard Methods (13). Table 1 lists other detailed operating conditions of ozonation.

The mono-chlorophenols used in this study were 2-chlorophenol, 3-chlorophenol and 4-chlorophenol. They were obtained from Merck (reagent grade). The initial concentrations of the test CPs were set at 100 mg/l (0.78 mM) and 200 mg/l (1.56 mM). Since the ozone dosage applied was maintained at 1.57 mmol/min ($q \times [O_3]_{in} = 0.976\,L/min \times 77.0\,mg/l = 75.2\,mg/min$), the ratio values of compound concentration (mM)/ozone dosage (mmol/min) was 1 and 0.5. The volume of reaction solution is 1000 ml and Milli-Q water was used in all experiments. The pH was fixed at pH 7 in all ozonation. A pH controller (SUNTEX, pH/ORP Controller PC-310), an adding pump (Cole-Parmer 7553-80) and 0.2 N NaOH or H_2SO_4 solution was utilized to control pH during ozonation. Next, the color formation during ozonation was measured by a UV/VIS spectrophotometer (Metertek SP-830) and observing the absorbance at 420 nm.
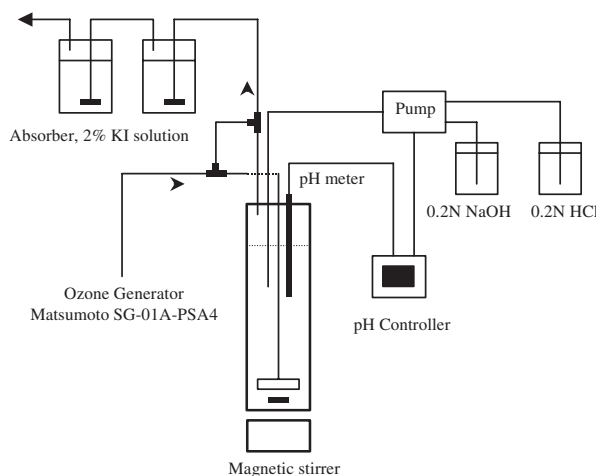

Figure 1. Assemblage of ozonation system.

Table 1.   Operating Conditions of Ozonation

Gas Phase	
Flow rate, q	976 ml/min
Pressure	1 kg/cm^2
Influent ozone concentration $[O_3]_{in}$	77.0 mg/l
Ozone dosage applied, $q \times [O_3]_{in}$	75.2 mg/min (1.57 mmol/min)
Liquid Phase	
Bubble column reactor	327 mm high, 70 mm diameter
Volume of solution	1000 ml
Initial reaction concentration	100 mg/l (0.78 mM) 200 mg/l (1.56 mM)
pH condition	7

Analytical Methods and Equipments

The CPs concentration and their intermediates during ozonation were analyzed by HPLC (Bio-Rad 1350) equipped with a UV/VIS spectro-photometer (Bio-Rad 1706) employing wavelength at 280 nm and a C18 reverse-phase column (Phenomenex). The mobile phase consisted of aceto-nitrile, water and acetic acid, regulated as 580 : 420 : 5.7 by volume ratio. A Finnngan TSQ-700 liquid chromatograph (LC) with mass spectrometry (MS) and an electrospray ionization (ESI) system was employed to identify the intermediates. The concentration of chloride ion was measured by using a Dionex 2000i ion chromatograph (IC) with a Dionex AS4A anion separator column. A TOC analyzer (O-I-Analytical, model 700) was employed to measure the total carbon concentration (TOC) of ozonated solution.

Microtox Toxicity Test

Acute toxicity of the pure compounds and solutions of their ozonated intermediates was measured by the Microtox toxicity technique, using *Photobacterium phosphoreum*. Analysis was according to the standard Microtox test procedures using a Microtox Analyzer (Model 500, Microbics Corp, Carlsbad, CA). In this paper, the $EC_{50,15\,min}$ value (that is the effective concentration of the sample that causes 50% inactivation of the test bacteria in 15 min) was used to trace the change in toxicity during ozonation. The Microtox test was operated in duplicate with a resulting average CV (Coefficient of Variation) of 15%. The pH value of all test samples was maintained at 7 to prevent the pH-effect, before toxicity was measured. The microorganism used in the Microtox produces light with peak intensity

at 490 nm. Consequently, highly colored aqueous samples may cause non-specific reductions in light level when analyzed according to the standard Microtox assay procedure. Any light level reduction could not be distinguished from one caused by toxicants. Therefore, a color correction procedure was incorporated in this study. The Microtox test of all samples was required to finish the measurement within 48 h after sampling, and the samples must storage under $4°C$ environment.

The EC_{50}-values expressed as TOC ($EC_{50, as \%} \times TOC$) are used in this study. In order to further discuss the contribution of toxicity for ozonated intermediates, EC_{50} values are transformed to toxicity units (TU) according to the approach introduced by Brown (14). Toxicity unit values was defined as following equation: $TU_i = C_i/EC_{50,i}$, where C_i is the concentration of component i. In addition, based on the concept of concentration additivity, the TU values could be summed up: $TU_{total} = \sum TU_i$ (14,15). Based on this, the toxicity unit values were summed up as $TU_{total} = TU_{CPs} + TU_{intermediates}$ in this study. The TU_{total} are calculated according to the TOC value and defined as $TOC/EC_{50,as\,TOC}$, while the TU_{CPs} are defined as $C_{CPs}/EC_{50,CPs.}$ Herein, CPs concentration in ozonated intermediates was measured using HPLC analysis. Based on the above principles, the toxicity unit of ozonated intermediates could be calculated from previously definition of TU additive.

RESULTS AND DISCUSSION

Variation of Toxicity for Ozonated Chlorophenols

Figure 2 summarized the results of Microtox EC_{50} as TOC (mg/L) in the ozonation of 2-CP, 3-CP and 4-CP under pH 7 solution. According to the figure, the results revealed that ozonation of CPs significantly induced new aqueous toxicity under various conditions. Namely, the EC_{50} value of unozonated CPs was higher than the minimum EC_{50} value of ozonated intermediates. This increasing toxicity normally reached their peak during ozonation. The variation of EC_{50} curve kept its downward trend after reaction started until the minimum value EC_{50} was hit, and further oxidation increased gradually the EC_{50} value into a non-toxic condition (non-toxic solution was defined as Microtox EC_{50} was over 100% in this study). Herein, the reaction concentration of CPs did not affect this phenomenon of increasing toxicity. Regarding the timing of maximum toxicity occurred, it generally happened at early stage of ozonation whether the concentration of CPs was high or low conditions. According to the results in Table 2, maximum toxicity could be observed at the moment when the ozone dosage applied did not exceed the initial amount of CPs. Namely, this increasing toxicity could reach its highest value when the ozone dosage applied was within 1 mg of ozone per mg of initial CPs.
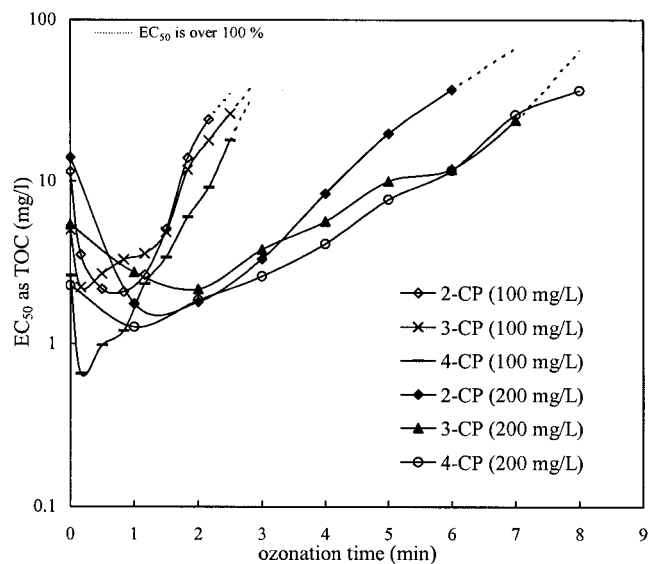
Figure 2. The variation of EC_{50} as TOC with time during the ozonation of 2-CP, 3-CP and 4-CP (100 and 200 mg/l) in pH 7 condition.

Table 2. Toxicity and Ozone Dosage Applied Results of Ozonation of Chlorophenols

		2-CP		3-CP		4-CP	
Concentration (mg/l)		100	200	100	200	100	200
EC_{50} as TOC (mg/l)	$t_o{}^a$	11.39	13.98	5.0	5.4	2.62	2.27
	$t_M{}^b$	2.08	1.76	2.22	2.17	0.653	1.27
TU_{total}	t_o	4.0	7.5	10.6	20.4	20.5	46.9
	t_M	20.4	57.4	23.2	47.4	80.2	81.5
O_3 applied	t_M	0.62	0.38	0.13	0.75	0.13	0.38
(O_3, mg/initial	Detoxify	>1.63	>2.25	>1.88	>2.63	>1.88	>3.01
CPs, mg)	into non-toxic						

[a]t_o: unozonated period.
[b]t_M: ozonated period with maximum toxicity.

In addition, 2-CP showed higher degree of toxicity increase than 3-CP or 4-CP during ozonation in low or high CPs concentration. The maximum TU value of ozonated 2-CP intermediates was 7.7 times to unozonated 2-CP solution (TU, $t_o = 7.5 \rightarrow$ TU, $t_M = 57.4$) in 200 mg/l concentration, while the ozonated 3-CP and 4-CP was 2.3 and 1.7 times to unozonated solutions, respectively (Table 2). This increasing TU value level of ozonated 2-CP and 3-CP under 100 mg/l concentration were very similar to those of the respective ozonated 200 mg/l concentration. However, the maximum TU

value of ozonated 4-CP was 3.9 times as high as unozonated solution in low reaction concentration; this increasing level was higher than in ozonated high concentration.

After maximum toxicity occurred, ozonation of 4-CP reduce the aqueous toxicity back to the original toxicity level faster than others, but it required largest ozone dosage to become the complete non-toxic solution among these ozonated CPs. However, ozonated 2-CP required more ozone dosage to detoxify the toxic intermediates back to original toxicity level, and needed less ozone treatment to become a non-toxic solution than 3-CP and 4-CP (Figure 2). Regarding ozone dosage requirement for detoxifying into non-toxic solution under 200 mg/l concentration, oxidized 4-CP, 3-CP and 2-CP used about 3.0, 2.6 and 2.3 mg of ozone per mg of initial CPs, respectively (Table 2). In addition, ozone needed of oxidizing 4-CP, 3-CP and 2-CP in 100 mg/l concentration for detoxifying the toxicant into a complete non-toxic solution was about 1.9, 1.9 and 1.6 mg of ozone per mg of initial CPs, respectively, and this value was lower than ozonation in high CPs concentration. This result indicated that low CPs concentration or high ozone dosage applied might reduce the existence time of increasing toxicity. Summarizing previously study, ozone requirement of detoxification equaled to the original toxicity situation was following the order 2-CP > 3-CP > 4-CP, but if ozone treatment was considered for the non-toxic condition, the order of ozone required was 4-CP > 3-CP > 2-CP. The results of this order were closely corresponded to low and high concentration of CPs.

Toxic Contribution of Ozonated Intermediates

The toxicity unit (TU) of ozonated intermediates was calculated from the additivity of TU value as described earlier. In order to obtain the TU_{CPs} value, the purely CPs EC_{50} value used in this study was calculated from the average value of unozonated CPs solution under low and high concentration. The Microtox $EC_{50,15\,min}$ (mg/l) of three chlorophenols was 2-CP: 24.7, 3-CP: 9.62 and 4-CP: 4.6, respectively. These Microtox EC_{50} values of chlorophenols were close to those shown in the published studies (1,16).

The contributions of toxicity units of unozonated CPs or ozonated intermediates and the variation of water parameter were summarized in Figures 3–5. The purely CPs compounds were the only toxicity source before ozonation started. The toxicity of CPs accounted for only a little portion of the total toxicity when the original CPs was reacted into their intermediates, and the increase in TU value was contributed mainly from intermediate compounds. Namely, the by-products were the source of increasing toxicity during oxidized CPs by ozone. Regarding the relationship between toxicity and color formation, the maximum TU value normally occurred a
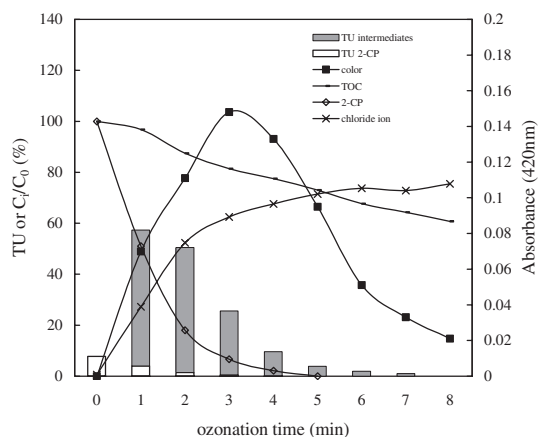
Figure 3. The variation of water parameter and toxicity unit of ozonated 2-CP (200 mg/l) with time in pH 7 condition.
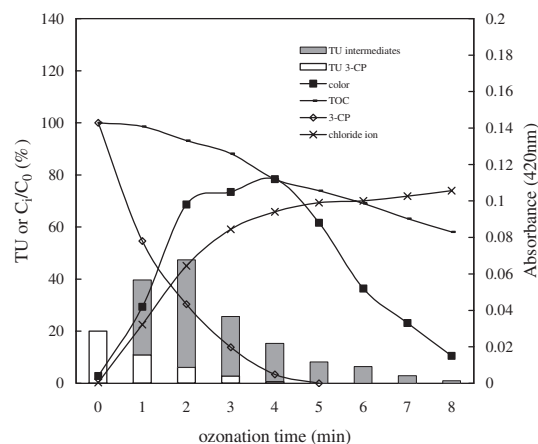

Figure 4. The variation of water parameter and toxicity unit of ozonated 3-CP (200 mg/l) with time in pH 7 condition.

little earlier than when the maximum color intensity expressed at 420 nm was monitored (Figures 3–5). Therefore, the colored matters might be one of the major intermediates of implying the new aqueous toxicity (17).

The aromatic compounds of containing chloro substituent might be the oxidation intermediates which increasing toxicity. According to the analysis of HPLC and mass spectrometry (MS), ozonated 2-CP, 3-CP and 4-CP formed chlorocatechols and chloromuconic acids as the major intermediates of chlorinated compound (18). Moreover, the intermediates of containing high molecular weights and only little concentration could also be monitored in this study. A compound of molecular weight 252 was detected in ozonated
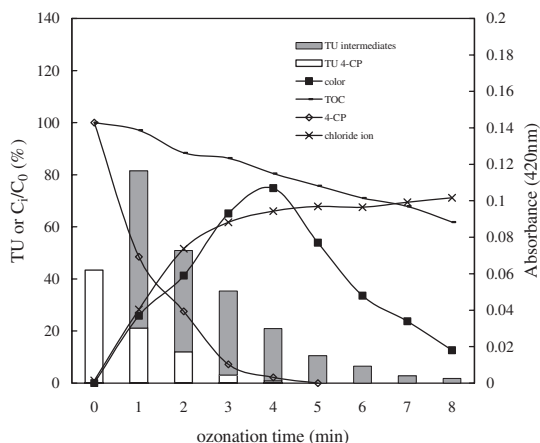
Figure 5. The variation of water parameter and toxicity unit of ozonated 4-CP (200 mg/l) with time in pH 7 condition.
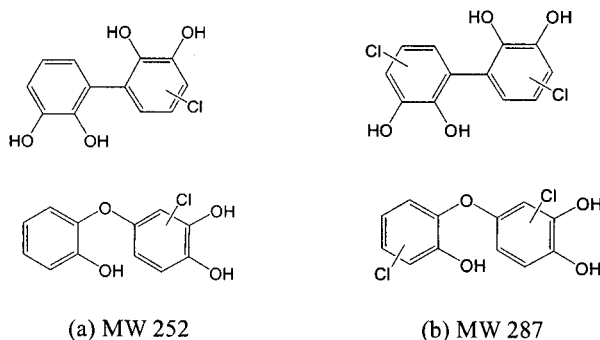

(a) MW 252   (b) MW 287

Figure 6. The dimeric structure compounds, ozonated intermediates of (a) 2-CP, (b) 3-CP or 4-CP.

2-CP, and a compound of molecular weight 287 was found in ozonated 3-CP or 4-CP. These high molecular weights compounds should contain hydroxylated/chlorinated dimeric structures (9,19), and the results are suggested in Figure 6. Hirvonen et al. (9) indicated that the hydroxylated or chlorinated dimeric intermediates were formed during ozonation of 2-CP and 4-CP in acidic solution, and chlorodihydroxylbiphenols (MW = 220) and dichlorodihydroxylbiphenols (MW = 254) were detected. Analysis of these dimeric compounds showed that the dimeric benzene ring was combined through the C–C bonding. In addition, Chrostowski et al. (19) also reported that oxidative coupling was the possible mechanism of forming dimeric structure; this reaction formed the C–O–C bonding to combine both benzene ring and form dimeric structure. Summarizing these findings, the results could not indicate very clearly which intermediates implied such increasing toxicity.

Nevertheless, the aromatic compounds with chlorinated or hydroxylated substitutes might be the oxidation intermediates that incurred an increasing toxicity during ozonation of chlorophenols.

CONCLUSIONS

This study investigated the variation of toxicity during ozonation of 2-CP, 3-CP and 4-CP in neutral condition. Results of Microtox assay showed that the ozonation of CPs induced new aqueous toxicity. Ozonation of 2-CP revealed higher level of toxicity increase than 3-CP and 4-CP. The maximum toxicity unit value of ozonated 2-CP intermediates was 7.7 times as high as that of unozonated 2-CP solution. In addition, this increasing toxicity normally reached its peak and a little earlier than when the maximum color intensity was monitored, while the ozone dosage applied was within 1 mg of ozone per mg of initial CPs. Namely, ozonation of chlorophenols could present the risk of toxic substances more than parent compounds during early stage of ozone treatment. The increasing toxicity was contributed mainly from ozonated intermediates. In this study, chlorocatechols, chloromuconic acids and formation of hydroxylated/chlorinated dimeric compounds were involved in ozonation of CPs. Therefore, further research is required to determine which compounds are major sources of increasing toxicity. Nevertheless, this investigation indicated that ozonation of CPs might generate new toxicity problems if these toxic intermediates are not further oxidized. In addition, ozone requirement of detoxifying into a non-toxic solution was following the order 4-CP > 3-CP > 2-CP.

ACKNOWLEDGMENTS

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