Degradation mechanism of alachlor during direct ozonation and O_3/H_2O_2 advanced oxidation process

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ABSTRACT

The degradation of alachlor by direct ozonation and advanced oxidation process O_3/H_2O_2 was investigated in this study with focus on identification of degradation byproducts. The second-order reaction rate constant between ozone and alachlor was determined to be $2.5 \pm 0.1 \text{ M}^{-1} \text{ s}^{-1}$ at pH 7.0 and 20 °C. Twelve and eight high-molecular-weight byproducts (with the benzene ring intact) from alachlor degradation were identified during direct ozonation and O_3/H_2O_2 , respectively. The common degradation byproducts included *N*-(2,6-diethylphenyl)-methylethylamine, 8-ethyl-3,4-dihydro-quinoline, 8-ethyl-quinoline, 1-chloroacetyl-2-hydro-3-ketone-7-acetyl-indole, 2-chloro-2',6'-diacetyl-*N*-(methoxymethyl)acetanilide, 2-chloro-2'-acetyl-6'-ethyl-*N*-(methoxymethyl)-acetanilide, and two hydroxylated alachlor isomers. In direct ozonation, four more byproducts were also identified including 1-chloroacetyl-2,3-dihydro-7-ethyl-indole, 2-chloro-2',6'-ethyl-acetanilide, 2-chloro-2',6'-acetyl-acetanilide and 2-chloro-2'-ethyl-6'-acetyl-*N*-(methoxymethyl)-acetanilide. Degradation of alachlor by O_3 and O_3/H_2O_2 also led to the formation of low-molecular-weight byproducts including formic, acetic, propionic, monochloroacetic and oxalic acids as well as chloride ion (only detected in O_3/H_2O_2). Nitrite and nitrate formation was negligible. Alachlor degradation occurred via oxidation of the arylethyl group, *N*-dealkylation, cyclization and cleavage of benzene ring. After O_3 or O_3/H_2O_2 treatment, the toxicity of alachlor solution examined by the *Daphnia magna* bioassay was slightly reduced.

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1. Introduction

Alachlor (2-chloro-2',6'-diethyl-*N*-(methoxymethyl)acetanilide) is among the most widely used pre-emergence herbicides all over the world (Mangian et al., 1997; Graham et al., 1999; Hladik et al., 2005a). Due to its extensive usage and moderate persistence, both alachlor and its metabolites could be accumulating in agriculturally related waters and the peak concentrations for alachlor of $25 \mu\text{g L}^{-1}$ in Kansas River and $4.8 \mu\text{g L}^{-1}$ in US groundwater were reported (Potter and Carpenter, 1995; Galassi et al., 1996; Thurman et al., 1996; Scribner et al., 2000; Squillace et al., 2002; Rebich et al., 2004). Concerns have been rising regarding the health risks associated with its occurrence in natural waters because alachlor is toxic and mutagenic (Tessier and Clark, 1995). To avoid potential human exposure to alachlor via drinking water, US EPA has set a maximum contaminant level (MCL) of $2.0 \mu\text{g L}^{-1}$ (US EPA, 2001), and European Union has even more strictly regulated an MCL for any particular pesticide at $0.1 \mu\text{g L}^{-1}$ and the sum of all pesticides

at $0.5 \mu\text{g L}^{-1}$, including their degradation byproducts (European Union, 2007).

Once alachlor emerges in source water with a concentration above the regulated MCL, appropriate water treatment processes have to be applied to comply with the drinking water standards. However, conventional unit operations for drinking water treatment such as pre-oxidation by permanganate, coagulation, filtration and chlorination show low removal efficiency for alachlor (<10%) (Miltner et al., 1987, 1989; Hladik et al., 2005b). The application of ozone for disinfection and oxidation of drinking water is widespread all over the world. However, conventional ozonation process at water plants could not provide a complete removal of alachlor, generally achieving a removal efficiency of about 63% (Verstraeten et al., 2002; Hladik et al., 2005b). The complete degradation of alachlor only occurred at higher O_3 dosages (Beltrán et al., 1999a).

The second-order rate constant of alachlor with molecular ozone is relatively low (Yao and Haag, 1991), while that with $\cdot\text{OH}$ is up to the diffusion-controlled rate (Haag and Yao, 1992). Therefore, advanced oxidation process (AOP) which generates abundant $\cdot\text{OH}$ has a great efficacy for the elimination of alachlor (Beltrán et al., 1999b). The combination of O_3 with H_2O_2 is the most com-

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monly used AOP at water plants (von Gunten, 2003). The reaction is described as follows:

For drinking water treatment, complete alachlor mineralization is often not feasible. Therefore, the transformation of alachlor during direct ozonation and AOP $\text{O}_3/\text{H}_2\text{O}_2$ would yield many degradation byproducts. Concerns were raised regarding the byproducts formed since sometimes pesticide degradates were equally or even more toxic than their parent compound (e.g., dichlorvos) (Liu et al., 2009). However, until now the identification of alachlor degradation byproducts during direct ozonation and AOP $\text{O}_3/\text{H}_2\text{O}_2$ is limited, and related degradation pathways are not clear.

With this context in mind, the major objectives of this study were to: (1) identify high-molecular-weight (HMW) degradation byproducts (i.e., byproducts with the benzene ring intact); (2) quantify the formation of low-molecular-weight (LMW) byproducts (i.e., byproducts without the benzene ring), namely small organic acids and inorganic anions; and (3) propose the degradation pathways of alachlor by O_3 and $\cdot\text{OH}$. In addition, the degradation kinetics of alachlor and the toxicity variation of alachlor solution during O_3 and $\text{O}_3/\text{H}_2\text{O}_2$ treatment were examined.

2. Materials and methods

2.1. Standards and reagents

Alachlor (99%), propachlor (99%) and peroxidase from horseradish (Type II, lyophilized, 5KU) were purchased from Sigma–Aldrich. *N,N*-diethyl-*p*-phenylenediamine (analytical grade) was purchased from Alfa Aesar. Sodium acetate, propionic acid and potassium chloride (analytical grade) were provided by Sinopharm Chemical Reagent Beijing Co., Ltd. Indigo and sodium formate were purchased from Tianjin Institute of Jingke Fine Chemicals. Sodium oxalate and monochloroacetic acid were purchased from Tianjin Chemical Reagent No. 1 Plant. Other chemicals were at least of analytical grade and used without further purification.

2.2. Analytical methods

Alachlor was determined at 230 nm by high-performance liquid chromatograph (HPLC, Agilent 1200) coupled with a diode array detector. Separation was performed by a Waters Atlantis column (3 μm , 150 $\times$ 2.1 mm) with gradient elution at 0.2 mL min^{−1}. The mobile phase consisted of HPLC-grade water (A) and acetonitrile (B) and the gradient elution was performed as follows: started from 70% A and 30% B, held for 5 min; linearly ramped to 50% A and 50% B over 5 min, held for 12 min; and further ramped to 70% A and 30% B over 3 min, held for 5 min. Sample injection volume was 50 μL , and the quantification limit for alachlor was 50 $\mu\text{g L}^{-1}$.

The concentration of residual O_3 was analyzed by the Indigo method (Bader and Hoigné, 1980). The concentration of H_2O_2 was determined by the peroxidase-DPD method (Bader et al., 1988). Total organic carbon (TOC) was analyzed with a Tekmar–Dohrmann Pheonix 8000 TOC analyzer.

LMW organic acids and inorganic anions were quantified by a Dionex 2000 reagent free ion chromatograph (IC) with an online eluent generator. Samples, injected via a 200 μL loop, were eluted at 1 mL min^{−1} through an Ionpac AS11-HC 4 mm column with the following gradient: 2 mM KOH, held for 8 min; increased to 15 mM KOH over 10 min; and further increased to 30 mM KOH over 12 min; held for 5 min. The retention times of acetic acid, propionic acid, formic acid, monochloroacetic acid, chloride, nitrite, nitrate and oxalate acid were 9.3, 10.8, 12.2, 16.3, 18.0, 19.8, 28.5 and

30.2 min, respectively. Their calibration curves in the concentration range from 1 to 50 μM showed a good linearity ($R^2 > 0.99$).

2.3. Experimental

2.3.1. Degradation of alachlor

The oxidation of alachlor by O_3 and $\text{O}_3/\text{H}_2\text{O}_2$ was first carried out in a batch reactor to determine the degradation kinetics by varying initial alachlor concentration and temperature. Ozone stock solutions were prepared by sparging ozone-containing oxygen produced with an ozone generator (Model CF-G-3-010g, Qingdao Guolin Ozone Equipment Co., China) into a receiving solution (i.e., Milli-Q water buffered with 10 mM phosphate to pH 7.0). The aqueous ozone concentration in the stock solution was monitored with Hach DR5000 spectrophotometer at 258 nm ($\epsilon = 3000 \text{ M}^{-1} \text{ cm}^{-1}$).

To determine the degradation kinetics of alachlor by molecular O_3 , the reaction was performed at pH 7.0 (10 mM phosphate buffer) and 10–26 °C in Milli-Q water. *tert*-Butyl alcohol (*t*-BuOH, 5 mM) was added to scavenge $\cdot\text{OH}$ formed from O_3 decomposition. The reaction was initiated by injecting 5–10 mL of the fresh alachlor solution (50 mg L^{−1}) into 100 mL of ozone stock solution. Samples were withdrawn at pre-selected time intervals to determine the residual ozone and alachlor concentrations. For alachlor analysis, residual ozone was first quenched with sulfite.

AOP $\text{O}_3/\text{H}_2\text{O}_2$ experiments were performed at pH 7.0 and 10 °C. The reaction was initiated by adding 4 mL of ozone solution with different initial concentrations (0–7.3 mg L^{−1}) to 4 mL of alachlor solution containing 0.4 mM H_2O_2 . After total ozone consumption (approximately within 5 min), the samples were analyzed by HPLC. Due to the low reactivity of alachlor with molecular O_3 (Yao and Haag, 1991), $\cdot\text{OH}$ was probably the predominant oxidant for alachlor degradation in $\text{O}_3/\text{H}_2\text{O}_2$.

2.3.2. Identification of HMW degradation byproducts

Solid-phase extraction was applied prior to the analysis and identification of HMW byproducts. Each reaction sample was extracted using a 500-mg Agilent SampliQ C18 extraction cartridge. The cartridge was conditioned with 5 mL of methanol and then 5 mL of distilled water. After passage of 100 mL of sample at a rate of approximately 60 drops min^{−1}, the cartridge was vacuum-dried and eluted with 4 mL of dichloromethane and 4 mL of methanol successively. The extracts were concentrated with a light stream of nitrogen gas to a final volume of 250 μL .

GC/MS (Agilent 7890 GC and 5975 MSD) coupled with an HP-5 MS column (30 m $\times$ 0.25 mm $\times$ 0.25 μm film thickness) was employed to analyze HMW byproducts with low polarity. Helium gas was used as carrier gas at a flow rate of 1 mL min^{−1}. The oven temperature started at 60 °C and held for 1 min; ramped linearly to 260 °C at 4 °C min^{−1} and held for 1 min; and further increased to 280 °C at 10 °C min^{−1}. The MSD was operated in the electron ionization mode at 70 eV.

Liquid chromatography/hybrid quadrupole time-of-flight mass spectrometry (LC/Q-TOF MS/MS) (Waters Micromass) was used for the identification of polar byproducts. The chromatographic conditions were as same as those aforementioned for determination of alachlor with HPLC. The HPLC was connected to a TOF mass spectrometer with an electrospray interface operated under the following conditions: capillary voltage 3.50 kV, cone voltage 20 V, source temperature 120 °C, desolvation temperature 300 °C, and collision energy 5 eV. Accurate mass measurements (four decimal places) were carried out at a resolution higher than 5000 (full width at half maximum) using an independent reference spray via the LockSpray interference to ensure accuracy. Propachlor was used as the internal lock mass with $[\text{M} + \text{H}]^+ = m/z$ 212.0842. All MS data were handled with MassLynx version 4.0.

2.3.3. Quantification of LMW organic acids and inorganic anions

LMW organic acids and inorganic anions formed during degradation of alachlor by O_3 and O_3/H_2O_2 were analyzed by IC. Experiments were conducted at 15 °C and an initial pH of 7.6. The oxidation of alachlor by O_3 in the presence of 5 mM *t*-BuOH was initiated by addition of 10 mL of fresh alachlor solution into 100 mL of ozone solution. The initial concentrations of ozone and alachlor were 14.1 mg L⁻¹ and 15.3 μM, respectively. Samples were withdrawn at pre-selected time intervals and the reaction was stopped by purging the residual ozone with nitrogen gas within 5 min. The samples were thereafter analyzed by HPLC and IC. O_3/H_2O_2 experiments were carried out by adding ozone solution (0–12.5 mg L⁻¹ concentration) to the alachlor solution containing 0.2 mM H_2O_2 . Samples were analyzed after ozone depletion.

2.3.4. Toxicity measurement

Daphnia acute immobilization tests were conducted following the national standards of China (Ministry of Environmental Protection of China, 1991). *Daphnia magna* was cultured in laboratory for more than three generations. Oxidation of alachlor by O_3 was conducted at 15 °C and pH 7.0 (10 mM phosphate buffer) with an initial concentration of 37 μM and 14.1 mg L⁻¹ for alachlor and ozone, respectively. Treated alachlor solution was tested after aqueous ozone depletion. Oxidation of alachlor by O_3/H_2O_2 was performed under the same conditions as ozonation except the addition of 0.2 mM H_2O_2 . After ozone depletion, 100 mg L⁻¹ Na_2SO_3 was added to quench the residual H_2O_2 . It is noted that at this level, Na_2SO_3 showed insignificant toxic effect on *D. magna*. Thereafter, the treated or untreated alachlor solution (20 mL) was added to 20 mL of synthetic natural water containing 10 *D. magna*. Test beakers with

D. magna neonates were incubated for 48 h at 20 °C in the dark. Acute toxicity was evaluated by monitoring the mobility of *D. magna*. The neonates were considered immobile if they remained at the bottom of the test beaker and did not resume swimming within the 15-s observation period. All the toxicity tests were performed in triplicate.

3. Results and discussion

3.1. Degradation kinetics of alachlor

Fig. 1 shows the degradation kinetics of alachlor by O_3 and O_3/H_2O_2 . In direct ozonation, 5.0 mM *t*-BuOH was added as to scavenge the $\cdot OH$ produced from ozone decay. It is estimated that about 99% of $\cdot OH$ could be scavenged by *t*-BuOH under the applied conditions (Acero et al., 2000). Fig. 1a shows the degradation of alachlor and the decay of O_3 as a function of reaction time. Alachlor reacted with molecular ozone slowly, exhibiting 84% removal after 60 min.

The overall reaction of ozone with organic compounds is generally of second-order, with first-order to each reactant (Hoigné and Bader, 1983; Yao and Haag, 1991). Hence, the degradation rate of alachlor can be expressed by the following equation:

$$\frac{-dC}{dt} = k[O_3]C \quad (2)$$

where k is the second-order rate constant and C denotes alachlor concentration. Rearranging and integrating Eq. (2) obtains:

$$\ln\left(\frac{C_0}{C}\right) = k \int [O_3] dt \quad (3)$$

Fig. 1. Degradation kinetics of alachlor: (A) decay of alachlor and O_3 during direct ozonation, $[alachlor]_0 = 5.5$ μM, $[O_3]_0 = 15.0$ mg L⁻¹, $[t\text{-BuOH}] = 5$ mM, $T = 20$ °C; (B) plot of $\ln(C_0/C)$ vs. CT during direct ozonation, $[t\text{-BuOH}] = 5$ mM, $T = 20$ °C; (C) Arrhenius plot of the reaction rate constants of alachlor with O_3 at different temperatures, $[t\text{-BuOH}] = 5$ mM; and (D) O_3/H_2O_2 at various O_3 dosages, $[H_2O_2]_0 = 0.2$ mM, $T = 10$ °C. Experimental conditions: pH = 7.0. The standard deviation of all data points was below 5%.

where the time-integrated ozone concentration, $\int[\text{O}_3]dt$, is the ozone exposure (i.e., CT). Fig. 1a shows the first-order kinetics for ozone decay, thus the CT value at any reaction time could be calculated. As depicted in Fig. 1b, a plot of $\ln(C_0/C)$ vs. CT gave the slope (k) value of $2.5 \pm 0.1 \text{ M}^{-1} \text{ s}^{-1}$ at 20°C and pH 7.0. It is noted that this value was somewhat less than $3.8 \pm 0.4 \text{ M}^{-1} \text{ s}^{-1}$ at 21°C reported by Yao and Haag (1991) who monitored ozone decay as a function of reaction time in the presence of at least 5-fold excess of alachlor. Ozone is unstable in water. Besides its reaction with target compound, ozone loss can also occur via other means (e.g., self-decomposition, volatilization and chain reactions initiated by substrates). Therefore, monitoring ozone decay rate tends to overestimate the reaction rate constant between ozone and the target compound. In fact, Yao and Haag (1991) also found that the reaction rate between atrazine and ozone obtained from monitoring ozone decay was faster than that obtained from monitoring atrazine decomposition. The rate constant determined by monitoring contaminant loss usually reflects more closely the rate of contaminant removal in an actual treatment process.

Alachlor is non-ionizable and hence the determined rate constant for the reaction between molecular ozone and alachlor is independent of pH. The effect of temperature on the reaction rate constant was investigated from 10 to 26°C . The Arrhenius plot shows an activation energy of 54 kJ mol^{-1} (Fig. 1c) which is close to the typical range of $35\text{--}50 \text{ kJ mol}^{-1}$ for molecular ozone reactions (Hoigné and Bader, 1983).

Conventional ozonation process can not provide effective control of alachlor. To remove alachlor at typical ozone dosages, the addition of H_2O_2 is usually required to enhance the generation of $\cdot\text{OH}$. Alachlor reacts very quickly towards $\cdot\text{OH}$ with a second-order rate constant of $7 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$ (Haag and Yao, 1992). Fig. 1d shows that the removal efficiency of alachlor ($C_0 = 10 \mu\text{M}$) reached 94% at $2.0 \text{ mg L}^{-1} \text{ O}_3$ dosage in the presence of $0.2 \text{ mM H}_2\text{O}_2$. The combination of H_2O_2 with O_3 could evidently enhance alachlor degradation. If the initial concentration of alachlor was increased, ozone dosage should be correspondingly raised to achieve a complete removal of alachlor (Fig. 1d).

Elovitz and von Gunten (1999) proposed a R_{ct} concept that was defined as the ratio of $\cdot\text{OH}$ to O_3 exposure during ozonation process. R_{ct} is typically in the range of $10^{-7}\text{--}10^{-9}$ in various natural waters. Therefore, the fraction of alachlor degraded by $\cdot\text{OH}$ (f_{OH}) can be expressed as follows:

$$f_{\text{OH}} = \frac{k_{\text{OH}}R_{\text{ct}}}{k_{\text{OH}}R_{\text{ct}} + k_{\text{O}_3}} \quad (4)$$

With the determined k_{O_3} value of $2.5 \text{ M}^{-1} \text{ s}^{-1}$ at 20°C and the literature-reported k_{OH} value of $7 \times 10^9 \text{ M}^{-1} \text{ s}^{-1}$, f_{OH} is calculated to be greater than 74% for ozonation of alachlor in natural waters. Due to the low reactivity of alachlor with molecular ozone, the indirect oxidation with $\cdot\text{OH}$ plays a major role for alachlor degradation during ozonation of drinking water.

3.2. Identification of HMW degradation byproducts

Fig. 2 shows the typical GC/MS chromatograms of the samples treated by direct ozonation and $\text{O}_3/\text{H}_2\text{O}_2$. The peaks assigned to Arabic numbers were alachlor (i.e., compound 11) and its HMW degradation byproducts. The peaks not assigned to any Arabic number were found to be most probably irrelevant to the degradation byproducts of alachlor after scrutinizing their mass spectra. Results indicate that direct ozonation of alachlor gave rise to 13 byproducts, while the oxidation of alachlor by $\cdot\text{OH}$ produced seven byproducts. The mass spectra of compounds 1–14 are compiled in Fig. 3 where the chemical structures of most byproducts (except compounds 3, 6 and 9) were identified. The mass spectra of byproducts were compared with literature information where available.

Compound 1 with retention time (RT) of 16.1 min and molecular weight (MW) of 161 could correspond to *N*-(2,6-diethylphenyl)-methylethylamine. It has a parent ion at m/z 161 and an abundant ion at m/z 146 with the loss of CH_3 in the ethyl group. The peaks agreed well with the mass spectrum reported previously (Potter and Carpenter, 1995; Mangiapan et al., 1997). This compound was detected as a degradation byproduct of alachlor in natural waters (Potter and Carpenter, 1995).

Compound 2 with RT 17.1 min and MW 159 could correspond to 8-ethyl-3,4-dihydro-quinoline. It has a parent ion at m/z 159 and an abundant ion at m/z 144 with the loss of CH_3 in the ethyl group. This compound was not previously reported as an alachlor degradate. The MW of compound 3 with RT 17.9 min was probably 161. The mass spectrum was similar with that of compound 1. However, its structure could not be attributed.

Comparing with the National Institute of Standards and Technology library, the probability of compound 4 (RT 19.6 min) being

Fig. 2. Typical GC/MS chromatograms of alachlor samples treated by direct ozonation and $\text{O}_3/\text{H}_2\text{O}_2$.

Fig. 3. MS spectra and proposed structures of degradation byproducts.

Fig. 3 (continued)

8-ethyl-quinoline is 91%. The molecular ion at m/z 157 could lose CH_3 in the ethyl group to give m/z 142. This compound was not previously reported as an alachlor degradate.

Compound **5** with RT 27.0 min and MW 223 could correspond to 1-chloroacetyl-2,3-dihydro-7-ethyl-indole. It has a parent ion at m/z 223 with the corresponding ^{37}Cl at m/z 225. The m/z 223 ion could lose CH_3 in the ethyl group to yield m/z 208. The spectrum of compound **5** is consistent with that of an alachlor biotransformation byproduct reported previously (Tiedje and Hagedorn, 1975; Wei and Vossbrinck, 1992).

Compound **6** with RT 28.8 min and MW 259 could not be assigned to any structure. However, the chlorine isotopic peak implied a chloroacetyl group in its structure. Compound **7** with RT 28.9 min and MW 225 could correspond to 2-chloro-2',6'-ethyl-acetanilide. It has a parent ion at m/z 225 with a chlorine isotopic ion at m/z 227, and an abundant ion at m/z 176 resulting from the loss of chloromethyl group. This compound was reported as an ala-

chlor metabolite formed in environment (Wei and Vossbrinck, 1992; Potter and Carpenter, 1995; Mangiapan et al., 1997).

Compound **8** with RT 30.7 min and MW 253 could correspond to 2-chloro-2',6'-acetyl-acetanilide. It has a parent ion at m/z 253 with the corresponding ^{37}Cl at m/z 255. The m/z 253 ion could lose the acetyl group to yield m/z 210. This compound was not reported before. Compound **9** with RT 32.3 min and MW 275 could not be assigned to any structure. The chlorine isotopic peak implied a chloroacetyl group in its structure, however.

Compound **10** with RT 32.7 min and MW 283 could correspond to 2-chloro-2'-ethyl-6'-acetyl-*N*-(methoxymethyl)acetanilide. The molecular ion at m/z 283 with the corresponding ^{37}Cl at m/z 285 could lose CH_2Cl in the chloroacetyl group to give m/z 234. This compound was not reported before.

Compound **11** with RT 34.0 min was alachlor which has a parent ion at m/z 269 with the corresponding ^{37}Cl at m/z 271. Compound **12** with RT 34.7 min and MW 251 could correspond to 1-

Table 1

Accurate mass measurement for alachlor and its degradation byproducts with LC/Q-TOF MS/MS.

Comp.	RT (min)	MW	Experimental mass (m/z)	Calculated mass (m/z)	$\Delta m\text{Da}$	ppm error	DBE ^a	Formula
I	7.9	253	276.0430	276.0403	2.7	9.6	6.5	$\text{C}_{12}\text{H}_{12}\text{NO}_3\text{ClNa}$
			254.0586	254.0584	0.2	0.8	6.5	$\text{C}_{12}\text{H}_{13}\text{NO}_3\text{Cl}$
II	9.3	297	320.0660	320.0666	-0.6	-1.7	6.5	$\text{C}_{14}\text{H}_{16}\text{NO}_4\text{ClNa}$
			298.0857	298.0846	1.1	3.7	6.5	$\text{C}_{14}\text{H}_{17}\text{NO}_4\text{Cl}$
			266.0586	266.0584	0.2	0.8	7.5	$\text{C}_{13}\text{H}_{13}\text{NO}_3\text{Cl}$
III	13.7	285	308.1036	308.1029	0.7	2.1	4.5	$\text{C}_{14}\text{H}_{20}\text{NO}_3\text{ClNa}$
			286.1198	286.1210	-1.2	-4.2	4.5	$\text{C}_{14}\text{H}_{21}\text{NO}_3\text{Cl}$
			268.1109	268.1104	0.5	1.7	5.5	$\text{C}_{14}\text{H}_{19}\text{NO}_2\text{Cl}$
IV	14.6	285	308.1021	308.1029	-0.8	-2.7	4.5	$\text{C}_{14}\text{H}_{20}\text{NO}_3\text{ClNa}$
			286.1194	286.1210	-1.6	-5.6	4.5	$\text{C}_{14}\text{H}_{21}\text{NO}_3\text{Cl}$
			268.1111	268.1104	0.7	2.5	5.5	$\text{C}_{14}\text{H}_{19}\text{NO}_2\text{Cl}$
V	16.5	283	306.0880	306.0873	0.7	2.3	5.5	$\text{C}_{14}\text{H}_{18}\text{NO}_3\text{ClNa}$
			284.1064	284.1053	1.1	3.7	5.5	$\text{C}_{14}\text{H}_{19}\text{NO}_3\text{Cl}$
Alachlor	25.5	269	270.1248	270.1261	-1.3	-4.7	4.5	$\text{C}_{14}\text{H}_{21}\text{NO}_2\text{Cl}$
			238.0992	238.0999	-0.7	-2.8	5.5	$\text{C}_{13}\text{H}_{17}\text{NOCl}$

^a DBE: double-bond equivalency.

chloroacetyl-2-hydro-3-ketone-7-acetyl-indole. It has a parent ion at m/z 251 with the corresponding ^{37}Cl at m/z 253. The m/z 253 ion could lose CH_2Cl in the chloroacetyl group to give m/z 202, and completely lose the chloroacetyl group to give m/z 174. This compound was not reported before.

Compound **13** with RT 36.5 min and MW 297 might correspond to 2-chloro-2',6'-diacetyl-*N*-(methoxymethyl)acetanilide. It has a parent ion at m/z 297 with the corresponding ^{37}Cl at m/z 299. Similar to the fragmentation of alachlor molecular ion, the molecular ion of compound **13** at m/z 297 could lose CH_3OH in the methoxymethyl group to give m/z 265, and completely lose the methoxymethyl moiety to give m/z 252. This compound was not reported before.

Compound **14** with RT 37.2 min and MW 283 could correspond to 2-chloro-2'-acetyl-6'-ethyl-*N*-(methoxymethyl)acetanilide. The molecular ion at m/z 283 with a chlorine isotopic ion at m/z 285 could lose Cl to give m/z 248. The spectrum agreed with that reported previously (Jacobsen et al., 1991; Chiron et al., 1995). This compound was detected as an alachlor degradate in groundwaters (Potter and Carpenter, 1995).

For relatively polar HMW byproducts (if formed) that were difficult to detect by GC/MS, LC technique was generally preferred. The efficacy of the combination of LC/Q-TOF MS/MS and GC/MS techniques in identification of unknown byproducts has been demonstrated (Gómez et al., 2008). The LC/Q-TOF MS/MS chromatograms show that both direct ozonation and $\text{O}_3/\text{H}_2\text{O}_2$ yielded several degradation byproducts of alachlor (i.e., compounds I–V). Table 1 presents the measured and calculated masses of byproduct ions, the error between them, and the proposed empirical formula

of the compounds identified by LC/Q-TOF MS/MS. In addition to the information from the protonated molecular ion, the presence of sodium adducts also confirmed the analyte identity, thus the accurate mass information of characteristic fragments facilitated the structure assignment. It is noted that all the protonated molecular ions or the sodium adducts had their own corresponding ^{37}Cl isotopic ions for confirmation.

Based on the accurate mass of the protonated molecular ion of compound **I** (m/z 254.0586), the formula of $\text{C}_{12}\text{H}_{13}\text{NO}_3\text{Cl}$ was proposed with a low error (0.8 ppm). The fragmentation style was similar with that obtained by GC/MS for compound **8**, thus it was assigned as 2-chloro-2',6'-acetyl-acetanilide. Due to the formation of ketone group, the double-bond equivalency (DBE) which represents the number of rings and double bonds in the molecular ion of compound **I** was greater than that of alachlor (increased from 4.5 to 6.5).

The ion (m/z 320.0660) of compound **II** was a sodium adduct with the proposed formula of $\text{C}_{14}\text{H}_{16}\text{NO}_4\text{ClNa}$ (−1.7 ppm error). The protonated molecular ion at m/z 298.0857 (3.7 ppm error) could lose CH_3OH to yield m/z 266.0586 (0.8 ppm error). Compound **II** was assigned as 2-chloro-2',6'-diacetyl-*N*-(methoxymethyl)acetanilide. Its presence was also confirmed by the GC/MS spectrum of compound **13** which exhibited a similar fragmentation style. The structure of compound **II** was also verified by the increase of DBE in comparison with that of alachlor.

Compounds **III** and **IV** were isomers because they had the same fragment ions. The protonated molecular ion (m/z 286.1198) was proposed to have the formula of $\text{C}_{14}\text{H}_{21}\text{NO}_3\text{Cl}$ (−4.2 ppm error). The parent ion could lose H_2O to yield m/z 268.1109. The two isomers were assigned as hydroxylated alachlors with structures shown in Fig. 5.

Compound **V** with the protonated molecular ion at m/z 284.1064 was assigned to the formula of $\text{C}_{14}\text{H}_{19}\text{NO}_3\text{Cl}$ (3.7 ppm error). The fragmentation style was similar to that obtained in GC/MS for compound **14** (i.e., 2-chloro-2'-acetyl-6'-ethyl-*N*-(methoxymethyl)acetanilide). Compound **V** was an important byproduct of alachlor because it had the highest peak among the degradation byproducts in both GC/MS and HPLC chromatograms.

3.3. Formation of LMW organic acids and inorganic anions

The LMW organic acids (i.e., formic, acetic, propionic, monochloroacetic and oxalic acids) and inorganic anions formed during alachlor degradation were also identified and quantified (Fig. 4). It is noted that during the oxidation of alachlor, nitrite and nitrate formation was negligible, implying that the *N* group was stable.

During direct ozonation, small organic acids were immediately generated along with alachlor degradation. The formation of organic acids was fast during the initial 60 min, and then slowed down due to the decreased ozone concentration (Fig. 4a). These organic acids could directly result from the degradation of alachlor. Similar phenomenon was observed in $\text{O}_3/\text{H}_2\text{O}_2$ oxidation of alachlor. Fig. 4b shows that formic acid concentration continuously increased with ozone dose, even when alachlor was almost removed at an O_3 dose of 8.0–12.5 mg L^{-1} . Therefore, formic acid could be generated from degradation of either alachlor or its intermediates, demonstrating a higher oxidation potential of $\cdot\text{OH}$ than molecular O_3 .

Propionic acid was generated due to the breakdown of the aromatic ring. Formic, acetic and oxalic acids could be generated from either breakdown of the aromatic ring or dealkylation and further oxidation of the side chains. The loss of chloroacetyl group led to the formation of monochloroacetic acid, which was also identified as a biodegradation and photodegradation byproduct of alachlor (Mangiapani et al., 1997; Ruth and Mabury, 2000). In direct ozonation, the chlorine atoms in monochloroacetic acid accounted for

Fig. 4. Formation of organic acids and chloride ion during degradation of alachlor by: (A) direct ozonation, $[\text{alachlor}]_0 = 15.3 \mu\text{M}$, $[\text{O}_3]_0 = 14.1 \text{ mg L}^{-1}$, $[\text{t-BuOH}] = 5 \text{ mM}$; and (B) $\text{O}_3/\text{H}_2\text{O}_2$, $[\text{alachlor}]_0 = 19.8 \mu\text{M}$, $[\text{H}_2\text{O}_2]_0 = 0.2 \text{ mM}$. Experimental conditions: $T = 15^\circ\text{C}$, $\text{pH}_0 = 7.6$. Error bars represent the standard deviation of duplicate analyses.

*MCAA: monochloroacetic acid

Fig. 5. Proposed pathways for alachlor degradation by O_3 (solid lines) and $\cdot OH$ (dashed lines).

about 43% of the total chlorine initially present in alachlor when 89% of alachlor was degraded. In other words, approximately 48% of alachlor was degraded via the loss of the chloroacetyl group. In contrast, in O_3/H_2O_2 about 30% of alachlor was degraded via the loss of the chloroacetyl group by $\cdot OH$.

Chloride release during direct ozonation was insignificant. Ozone did not appear to remove chlorine atoms readily (Somich et al., 1988; Adams and Randtke, 1992). Rather than the selective attack of molecular ozone, $\cdot OH$ attacks the functional groups non-selectively. As a result, dechlorination of alachlor occurred in O_3/H_2O_2 . Qiang et al. (2008) also reported that dechlorination readily occurred during the oxidation of chlorinated aliphatic hydrocarbons by Fenton's reagent that generates abundant $\cdot OH$ as the primary oxidant. After complete degradation of alachlor by O_3/H_2O_2 , the released chloride ion accounted for about 33% of the total chlorine atoms initially present in alachlor (Fig. 4b). Besides monochloro-

roacetic acid and chloride, other chlorinated degradation byproducts of alachlor (compounds I–V included) only accounted for about 37%.

TOC removal was insignificant (<10%) in both direct ozonation and O_3/H_2O_2 . When the reaction was complete, the small organic acids accounted for about 21% and 26% of the initial TOC in direct ozonation and O_3/H_2O_2 , respectively. The majority of alachlor was degraded to various organic byproducts rather than being mineralized. Thus, the toxicity of treated alachlor solution should be concerned.

3.4. Proposed degradation pathways

Based on the above information, the degradation pathways of alachlor by O_3 and $\cdot OH$ are proposed in Fig. 5. Percentages were gi-

ven for the relative importance of a pathway (only for the degradation byproducts that were quantified).

In direct ozonation, the attack of molecular ozone on alachlor could occur on the ethyl, *N*-methoxymethyl, *N*-chloroacetyl groups or the benzene ring. The ethyl-side chain could be oxidized to an acetyl group by ozone to yield compound **14** (V) or compound **10**. Hapeman-Somich (1991) also suggested that the primary ozonation product of alachlor should be a compound with one of the ethyl chains converted to the acetyl group. Compound **14** (V) was also the principal product of oxidation of alachlor by permanganate, indicating that the ethyl chain is readily oxidizable. Further oxidation of the ethyl chain of compound **14** (V) or compound **10** would yield compound **13** (I). Besides the oxidation of the arylethyl group, cleavage of the *N*-methoxymethyl group is a significant feature of environmental degradation of alachlor (Potter and Carpenter, 1995). The *N*-dealkylation mechanism was previously reported for oxidation of atrazine by O_3 (Adams and Randtke, 1992; Acero et al., 2000). By analogy, compound **7** could also be generated during the ozonation of alachlor. Successively, both oxidation of the arylethyl group of compound **7** and *N*-dealkylation of compound **13** (I) would yield compound **8** (II). Cyclization was an important pathway in photodegradation and photocatalytic degradation of alachlor (Chiron et al., 1995; Wong and Chu, 2003). In this study, cyclization was initiated by *N*-dealkylation of alachlor to form compound **5**. Further oxidation of compound **5** or cyclization of compound **8** (II) gave rise to compound **12**.

The electrophilic attack of ozone on the benzene ring or the arylethyl group would produce compounds **III** and **IV** which were also detected during photocatalytic oxidation of alachlor (Wong and Chu, 2003). Somich et al. (1988) proposed that the benzene ring cleavage could occur during the degradation of alachlor by ozone, and thus formic, acetic, propionic and oxalic acids were generated. Likewise, the benzene ring cleavage of compounds **III** and **IV** could also lead to the formation of these organic acids.

Generally, dechlorination was not involved in direct ozonation. Ozone molecule cannot remove chlorine readily (Somich et al., 1988). However, the cleavage of the *N*-chloroacetyl moiety tended to occur and thus compound **1** and monochloroacetic acid could be generated, which accounted for about 48% of the total alachlor degradation as aforementioned. Further cyclization of compound **1** gave rise to compounds **2** and **4**.

In O_3/H_2O_2 , compound **14** (V) was generated via oxidation of the arylethyl group by $\cdot OH$. Further oxidation would yield compound **13** (I). After *N*-dealkylation and cyclization, compounds **8** and **12** were formed successively. By the direct addition of $\cdot OH$, compounds **III** and **IV** could be generated. Similar to that in direct ozonation, small organic acids were produced through the cleavage of benzene ring. About 30% of alachlor was degraded via cleavage of the *N*-chloroacetyl moiety, leading to the formation of compound **1** and monochloroacetic acid, and successively compounds **2** and **4** by cyclization. The observed continuous chloride release implies the dechlorination of alachlor upon $\cdot OH$ attack. 2-Hydroxy-2',6'-diethyl-*N*-(methoxymethyl)acetanilide was an expected byproduct formed via dechlorination. However, this compound was not detected probably due to the limitation of our analytical methods.

Compounds **2**, **4** and **12** seem more resistant to oxidation than alachlor according to their chemical structures. If their benzene ring could be broken down by O_3 or $\cdot OH$, the eventual formation of small organic acids would be expected.

3.5. Toxicity assessment

The inhibition values of alachlor solutions on the motility of the daphnids prior and after oxidation, examined by the *D. magna* bioassay, were $33.8 \pm 5.8\%$ (untreated), $23.3 \pm 5.8\%$ (O_3) and $26.7 \pm 11.5\%$ (O_3/H_2O_2). It is seen that after either O_3 or O_3/H_2O_2

oxidation, the toxicity of alachlor solution was slightly reduced. Upham et al. (1997) have also reported that ozonated aqueous solutions of alachlor were slightly less toxic to gap junctional intercellular communication than alachlor itself. The fact that the treated alachlor solution remained a similar toxicity raised the concern about the toxicity of degradation byproducts. Compound **7** was mutagenic (Tessier and Clark, 1995) and monochloroacetic acid was a suspected carcinogen. To safeguard the drinking water quality, additional research about the chronic dietary risk of alachlor degradates to human health should be addressed in the future.

4. Conclusions

This study investigated the degradation of alachlor by O_3 and O_3/H_2O_2 , particularly focusing on byproducts identification. The second-order rate constant and activation energy for the reaction between alachlor and molecular ozone was experimentally determined. The degradation pathways of alachlor by O_3 or O_3/H_2O_2 were proposed which mainly included the oxidation of arylethyl group, *N*-dealkylation, cyclization and cleavage of benzene ring. The toxicity of treated alachlor solutions was slightly reduced.

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