Does KMnO_4 preoxidation reduce the genotoxicity of disinfection by-products?

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HIGHLIGHTS

- Increasing KMnO_4 dosage decreased the genotoxicity of chlorinated BP-4.
- KMnO_4 first increased and then decreased the genotoxicity of Tyr after chlorination.
- After treatment, mixture genotoxicity was unequal to sum of individual genotoxicity.
- The decrease of DBPs genotoxicity need a proper dose of KMnO_4 preoxidation.

GRAPHICAL ABSTRACT

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ABSTRACT

Potassium permanganate (KMnO_4) preoxidation is capable of affecting the formation of disinfection by-products (DBPs). However, few studies have focused on the toxicity of DBPs after KMnO_4 preoxidation, which is an important index to evaluate alternative treatment processes. Herein genotoxicity (SOS/umu test) was used to clarify the impact of KMnO_4 preoxidation on the chlorination byproducts produced from two representative precursors, tyrosine (Tyr) and 2-hydroxy-4-methoxybenzophenone-5-sulfonic acid (BP-4), and their mixture. Results revealed that although KMnO_4 could not oxidize BP-4, after chlorination KMnO_4 could oxidize the chlorination byproducts of BP-4 and thus decrease the genotoxicity production. For Tyr, KMnO_4 preoxidation could increase or decrease the genotoxicity of DBPs, depending on the KMnO_4 dose. The optimal initial molar ratio of KMnO_4 to Tyr was confirmed to be 1:1. It has been proved that both the oxidation of Tyr by KMnO_4 and manganese dioxide (MnO_2 , the reduction product of KMnO_4) and the oxidation of chlorination byproducts by MnO_2 can decrease the genotoxicity production of chlorinated Tyr. Remarkably, during chlorination, the competition of manganese(II) oxidation with organic oxidation can result in less chlorine reacting with organics, to induce an increase in genotoxicity. This is the main cause for the increase in genotoxicity of chlorinated Tyr after KMnO_4 preoxidation. Additionally, the genotoxicity of the chlorinated mixture was shifted from being higher than the sum of individual genotoxicities of the chlorinated precursors to being lower than their sum with increasing KMnO_4 dosage, due to the combined effects between the preoxidation-chlorination products from the two compounds.

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1. Introduction

Freshwater resource shortages and growing water demand have

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spurred utilities to exploit source waters impaired by wastewater effluents and/or algal blooms. Pretreatment with preoxidants is commonly applied in such source waters to inactivate the algal cells and improve the removal of taste and odor, color, inorganics and dissolved organic matter (DOM) (Ma et al., 1997; Petruševski et al., 1996; Saunier et al., 1983). The efficient removal of DOM (the main precursor of disinfection by-products (DBPs)) by preoxidation will be beneficial for controlling the formation of DBPs. Potassium permanganate (KMnO₄) is one of the most versatile oxidants known, due to its good stability, ease of handling and effectiveness over a wide pH range (Sun et al., 2013; Jiang et al., 2011; Waldemer and Tratnyek, 2006; Wu et al., 2012). Previous studies have shown that the KMnO₄ preoxidation can affect the formation of DBPs by removing and/or chemically transforming precursors prior to chlorination (Chu et al., 2015; Jiang et al., 2014; Chen and Valentine, 2008). However, these studies did not measure the toxicological effects (an important index for evaluating alternative treatment processes). So the central issue of whether KMnO₄ preoxidation can eliminate/decrease the toxicity of DBPs is still unclear.

The composition of source waters impaired by wastewater and/or algal bloom is complicated, including natural organic matter (NOM, e.g., humic/fulvic acids, proteins and amino acids) anthropogenic chemicals (e.g., pesticides and personal care products) and salts (e.g., bromide and iodide). Many of these substances can react with chlorine and produce a variety of DBPs (Richardson, 2011; Yang and Zhang, 2016; Gunten et al., 2001; Kampioti and Stephanou, 2002; Richardson et al., 2007; Shen and Andrews, 2011; Siddiqui et al., 2000). Thus it is too difficult to study how KMnO₄ preoxidation affects precursors to control DBP toxicity in actual water. To simplify this problem, we chose two representative DBP precursors (one is NOM and the other is anthropogenic chemical) in this study (Chu et al., 2012; Smith and El-Deen, 2009; Trehy et al., 1986; Xiao et al., 2013). Humic/fulvic acids are important components of the NOMs. However, studying the transformation mechanisms of humic/fulvic acids during peroxidation and chlorination is quite difficult, due to their complicated chemical structures. Tyrosine (Tyr) with a simple structure is a natural amino acid and is present in many peptides, proteins and algae (Ram, 1985; Szajdak and Österberg, 1996). In some US water treatment plants influent samples, the Tyr concentration of influent may reach 27.4 $\mu\text{g L}^{-1}$ (Mitch et al., 2009). 2-Hydroxy-4-methoxybenzophenone-5-sulfonic acid (BP-4) is one of the most important organic UV filter compounds and is widely used in personal care products (e.g. sunscreens, cosmetics, skin lotions, hair sprays) (Díaz-Cruz et al., 2008; Rodil and Moeder, 2008; Roelandts et al., 1983). BP-4 can enter the aquatic environment through showering, washing, swimming and so forth (Lambropoulou et al., 2002; Poiger et al., 2004; Thomas et al., 2004). The concentration of BP-4 was ranged from ng L^{-1} to high $\mu\text{g L}^{-1}$ in wastewater, sea and river water (Rodil et al., 2008; Fent et al., 2010). Both Tyr and BP-4 are frequently detected in wastewater and/or algal blooms (Balmer et al., 2005; Jeon et al., 2006). Overall, they are common precursors in reclaimed source waters.

In our previous study, the genotoxicity of Tyr and BP-4 alone and in mixture with and without chlorination were investigated. Results demonstrated that after chlorination, the genotoxicity of Tyr, BP-4 and their mixture significantly increased due to the formation of DBPs. N-DBPs dominated the genotoxicity of chlorinated Tyr and chlorinated mixture. Remarkably, when the two compounds were mixed and then chlorinated, the genotoxicity was amplified at pH 6 (i.e., after chlorination, the genotoxicity of the mixture was significantly higher than the sum of their individual genotoxicity values). This was ascribed to the synergistic effect between DBPs from Tyr and BP-4. Here we hypothesized that the KMnO₄ can oxidize Tyr and/or BP-4, thus decreasing the genotoxicity of their respective

DBPs after chlorination as well as the amplified genotoxicity from their chlorinated mixture. Our study was carried out to verify this hypothesis and also address the following questions: Does there exist an optimal dose of KMnO₄ for eliminating/decreasing the genotoxicity of the chlorinated individuals/mixture? What is the role of KMnO₄ preoxidation in controlling DBP formation and decreasing the genotoxicity of Tyr/BP-4 individually and their mixture after chlorination?

2. Materials and methods

2.1. Chemicals and solution preparation

Tyr was purchased from Sigma-Aldrich (USA). BP-4 was obtained from J&K China Chemical Ltd. The KMnO₄ and the sodium hypochlorite aqueous solutions were obtained from Sinopharm Chemical Reagent Co., Ltd (China). The concentration of the chlorine stock solution was standardized using the N, N-Diethyl-*p*-phenylenediamine ferrous titration method. The KMnO₄ (100 mM) and manganese chloride (MnCl₂, 200 mg L^{-1}) stock solutions were prepared before each experiment. A stock solution of colloidal MnO₂ was prepared following the method of Murray (Murray, 1974) in Text S1 of Supplementary Data (SD). Unless otherwise noted, all solutions were prepared from reagent grade chemicals and water purified using a Millipore Purification System (ultrapure water, 18.2 $\text{M}\Omega\text{ cm}^{-1}$ conductivity).

2.2. Experimental procedures

For all experiments, the solutions without KMnO₄ preoxidation served as the control samples. One hundred micromolar acetate buffer was used to maintain pH = 6.0 ± 0.1 throughout the reactions. The experiments were conducted in four parts. In part 1, the preoxidation processes were carried out with 500 mL of Tyr (0.1 mM), BP-4 (0.1 mM) and their mixture (0.1 mM, [Tyr]₀: [BP-4]₀ = 8:2) in 1000 mL glass beakers, respectively. The KMnO₄ at the desired concentrations (molar equivalent ratios of [KMnO₄]₀: [precursor]₀ were 0:1, 1:10, 1:5, 1:2, 1:1 and 1.5:1) were dosed into the solutions with stirring at 200 rpm for 2 h. The samples were divided into two portions. One was subjected to genotoxicity determination and the other was subjected to chlorination. The chlorination experiments were initiated by adding 1.5 mM of free chlorine and performed under headspace-free conditions in glass screw-cap vials capped with Teflon-faced septa. The chlorinated samples were incubated at room temperature ($25 \pm 0.2\text{ }^{\circ}\text{C}$) in the dark for 24 h. In part 2, to investigate the effect of KMnO₄ on the genotoxicity production of chlorinated BP-4, different dosages of KMnO₄ were added to 250 mL of BP-4 (0.1 mM) during chlorination. To investigate the effect of the formed MnO₂ on the genotoxicity production of chlorinated Tyr, different concentrations of colloidal MnO₂ ([MnO₂]₀: [Tyr]₀ = 0:1, 1:10, 1:5, 1:2, 1:1 and 1.5:1) were added to 250 mL of Tyr (0.1 mM) before and during the chlorination. ($25 \pm 0.2\text{ }^{\circ}\text{C}$, $t = 24\text{ h}$). In part 3, after preoxidation, different dosages of chlorine ([Cl₂]₀: [precursor]₀ = 15:1, 20:1 and 30:1) were dosed into 0.1 mM of Tyr with and without KMnO₄ preoxidation and 0.1 mM of BP-4 at room temperature ($25 \pm 0.2\text{ }^{\circ}\text{C}$) for 24 h, respectively, to verify the effect of chlorine dosage on DBP genotoxicity. To verify the effect of the formed manganese(II) (Mn(II)) on the genotoxicity production, 250 mL of Tyr was chlorinated with different concentrations of Mn(II) (0.05 mg L^{-1} , 0.1 mg L^{-1} , 0.4 mg L^{-1} , 0.8 mg L^{-1} and 1.5 mg L^{-1}) for 24 h at room temperature ($25 \pm 0.2\text{ }^{\circ}\text{C}$). In part 4, to investigate the combined effects on the augmentation and reduction of genotoxicity for the chlorinated mixtures, 0.08 mM of Tyr and 0.02 mM of BP-4 were preoxidized by KMnO₄ ($25 \pm 0.2\text{ }^{\circ}\text{C}$, $t = 2\text{ h}$) and chlorinated ($25 \pm 0.2\text{ }^{\circ}\text{C}$, $t = 24\text{ h}$),

separately, and then were mixed (hereafter we refer to them as the post preoxidation-chlorination mixture) and subjected to genotoxicity test. All of samples were found to have measurable chlorine using the N, N-Diethyl-*p*-phenylenediamine ferrous titration method. Sodium hyposulfite was used to quench the residual chlorine before genotoxicity testing.

2.3. Analytical methods

2.3.1. Determination of Tyr concentration

The concentration of Tyr was determined using an Agilent 1290 (Agilent, USA) liquid chromatograph equipped with a Waters ACQUITY UPLC[®] HSS T3 column (2.1 mm × 100 mm × 1.8 μm), and identified by a QQQ (Agilent 6460, USA) tandem mass spectrometer. A volume of 10 μL was injected at a column flow rate of 0.3 mL min⁻¹. The UPLC peak was resolved using gradient elution by varying the ratio of a formic acid aqueous solution (0.1% (v/v), A) to a formic acid acetonitrile solution (0.1% (v/v), B). The gradient program was as follows: 0–10 min 0% B, 10–25 min 0% B to 20% B, 25–35 min 20% B to 60% B, 35–40 min 60% A to 100% B, 40–45 min 100% B, 45–45.1 min 100% B to 0% B, 45.1–50 min 0% B. The mass analyses used the positive ion mode (ESI⁺). The mass spectrometer was operated in both electrospray ionization mode and in multiple-reaction monitoring mode. The capillary and fragmentation voltages were set at 4 kV and 80 eV, respectively. High-purity nitrogen served as the dry gas and the nebulizing gas. The gas temperature and flow rate were set at 350 °C and 8 mL min⁻¹, respectively. The mass spectrometer was configured to admit the protonated molecules [M + H]⁺ at *m/z* 182.1, and monitor the product ions at *m/z* 136 and 91.

2.3.2. Determination of BP-4 concentration

The concentration of BP-4 was analyzed by a Waters high performance liquid chromatograph (HPLC) equipped with a Waters ACQUITY UPLC[®] BEH C18 column (2.1 mm × 50 mm × 1.7 μm), and identified by a tandem mass spectrometer (Waters, USA) (Rosario et al., 2008). A volume of 10 μL was injected at a flow rate of 0.2 mL min⁻¹. The mobile phase used for gradient elution consisted of (A) 5 mM of ammonium acetate aqueous solution and (B) methanol containing 5 mM of ammonium acetate. The gradient program was as follows: 0 min 5% B, 2 min 50% B, 4 min 0% B, 6 min 5% B and 7 min 5% B. The mass analyses used the negative ion mode (ESI⁻). The desolvation and MS source temperatures were maintained at 450 °C and 110 °C, respectively. MS/MS experiments were carried out with high purity argon gas. The desolvation and cone gas flows were set at 700 L h⁻¹ and 50 L h⁻¹, respectively. The MS capillary voltage was 3 kV. The mass spectrometer was configured to admit the protonated molecules [M - H]⁻ at *m/z* 307, and monitor the product ions at *m/z* 79.6 and 281.2.

2.3.3. Determination of KMnO₄ and manganese concentrations

The KMnO₄ concentration was measured using a U-3010 UV-vis spectrophotometer (Hitachi Co, Japan) equipped with 10 mm quartz cuvettes. The concentration of manganese was determined using an inductively coupled plasma/optical emission spectrometry (ICP-OES, Agilent 710 series, USA).

2.3.4. SOS/umu test

To examine the genotoxic responses to Tyr, BP-4 and their mixture after chlorination/peroxidation and chlorination, a modified SOS/umu test (Oda et al., 1985) for water samples was used. The test strain was the *Salmonella typhimurium* TA1535/pSK1002 without S9 activation. The details of the SOS/umu test were described in Text S2 of SD. Prior to genotoxicity testing, to eliminate the adsorption effect of MnO₂ toward organics, each 250 mL of

peroxidation-chlorinated sample was first acidified to pH = 2. The adjusted samples were concentrated by solid phase extraction (Oasis HLB cartridge, 6 mL, 500 mg, Waters, USA). The cartridge was previously washed with 5 mL of methylene dichloride, 5 mL of methanol and 5 mL of ultrapure water. Organics retained in the cartridge were eluted with methylene dichloride, methanol and acetone. The eluent was dried under a nitrogen flow in a water bath at 30 °C. The dried residuals were dissolved into 250 μL of dimethyl sulfoxide (DMSO) and stored at -20 °C in the dark. The concentrated water sample was subjected to the SOS/umu test, which was conducted in triplicate. During each test, 4-nitroquinoline-1-oxide (4-NQO) and DMSO were used as positive control and negative control, respectively. The equivalent 4-NQO concentration (TEQ_{4-NQO}) of water samples was calculated based on the dose-response curves of water samples and 4-NQO to quantitatively express the genotoxicity activity. More details were described in SD Text S2.

2.3.5. Statistical analysis

The means comparison and independent sample T-test were carried out using SPSS 19.0 (IBM). The means comparison was carried out to determine if the genotoxicity of chlorinated Tyr/BP-4/mixture was significantly changed with different dosages of KMnO₄ preoxidation. The T-test was used to determine if the genotoxicity of chlorinated mixtures was significantly different from the sum of the genotoxicity of individual chlorinated precursors after KMnO₄ preoxidation ([KMnO₄]₀: [precursor]₀ = 10:1, 5:1, 2:1, 1:1 and 1.5:1). The T-test was also carried out to determine if there were any significant genotoxicity differences between the post preoxidation-chlorination mixtures and the sum of the preoxidation-chlorination individual precursors.

3. Results and discussion

3.1. Comparison of genotoxicity production between KMnO₄ preoxidation-chlorination process and chlorination process

The preoxidation time was fixed at 2 h in the experiment, in which KMnO₄ showed a significant decrease in Tyr/mixture oxidation (Fig. S1(a) and (c)); but was almost unchanged throughout the experiment time in the BP-4 system (Fig. S1(b)). Accordingly, the genotoxicity production was compared between 2-h KMnO₄ preoxidation followed by 24-h chlorination, and 24-h chlorination alone. Results revealed that the genotoxicity reduction from BP-4 chlorination increased with increasing KMnO₄ dosages, while the genotoxicity production from Tyr chlorination was reduced by KMnO₄ preoxidation only with a proper dose (Fig. 1). The genotoxicity production of chlorinated Tyr after KMnO₄ preoxidation with [KMnO₄]₀: [Tyr]₀ = 1:10 and 1:5 was significantly higher than that without KMnO₄ preoxidation. A similar pattern was established in the genotoxicity decrease of the chlorinated Tyr and BP-4 mixture. There existed an optimal initial molar ratio of KMnO₄ to Tyr or Tyr/BP-4 mixture (1:1) to obtain a genotoxicity decrease. At this molar ratio, the genotoxicity reduction of chlorinated Tyr and their mixture reached at 47.5% and 71.6%, respectively. Based on a correlation analysis, the genotoxicity reduction of the chlorinated mixture was significantly related to that of chlorinated Tyr (*p* < 0.01, *r*² = 0.982) (Fig. S2). This is mainly because the nitrogen by-products (N-DBPs) produced from Tyr play a dominant role in the genotoxicity of the chlorinated mixture.

3.2. Reaction pathway

We first investigated the transformation pathways of BP-4 and Tyr by KMnO₄ oxidation and chlorination. Under the chosen experimental conditions, KMnO₄ could oxidize Tyr but had little

Fig. 1. The genotoxicity decrease of chlorinated by-products produced from (a) Tyr (0.1 mM) alone (b) BP-4 (0.1 mM) alone and (c) a Tyr and BP-4 mixture (0.1 mM, $[\text{Tyr}]_0 : [\text{BP-4}]_0 = 8:2$), respectively with different dosages of KMnO_4 preoxidation ($[\text{KMnO}_4]_0 : [\text{precursor}]_0 = 1:10, 1:5, 1:2, 1:1$ and $1.5:1$). The $\text{TEQ}_{4-\text{NQO}}$ values of Tyr, BP-4 and mixture with chlorination alone were $5.20 \pm 0.34 \text{ mg L}^{-1}$, $1.34 \pm 0.19 \text{ mg L}^{-1}$ and $5.82 \pm 0.47 \text{ mg L}^{-1}$ respectively. Error bars represent the standard deviation of triplicate tests. Preoxidation conditions: $t = 2 \text{ h}$, $\text{pH} = 6.0$ and $T = 25 \pm 0.2^\circ \text{C}$. Chlorination conditions: $t = 24 \text{ h}$, $\text{pH} = 6.0$ and $T = 25 \pm 0.2^\circ \text{C}$.

effect on BP-4 (Fig. S3). Concurrently, Mn(II) and MnO_2 were produced by the reduction of KMnO_4 . Based on these findings, the main reaction pathway of the mixture was deduced, as shown in Scheme 1. The first step (preoxidation) consisted of the oxidation of Tyr and the reduction of KMnO_4 (resulting in the formation of MnO_2 and Mn(II)). The formed MnO_2 may further react with the residual Tyr/intermediates. In the second step (chlorination), the

added chlorine and MnO_2 continue to react with residual precursors/intermediates. The Mn(II) formed during preoxidation can be adsorbed onto the reactive sites of the precipitated MnO_2 and oxidized by chlorine to form MnO_2 (Allard et al., 2013; Hao et al., 1991). This autocatalytic reaction competes with other oxidation reactions of residual precursors/intermediates with MnO_2 and chlorine.

Step 1: KMnO_4 preoxidation	Step 2: Chlorination
$\text{Tyr} + \text{KMnO}_4 \longrightarrow \text{A} + \text{MnO}_2 + \text{Mn(II)} \quad (1)$	$\text{A} + \text{Cl}_2 + \text{MnO}_2 \longrightarrow \text{C} + \text{Mn(II)} \quad (3)$
$\text{Tyr} + \text{MnO}_2 \longrightarrow \text{B} + \text{Mn(II)} \quad (2)$	$\text{B} + \text{Cl}_2 + \text{MnO}_2 \longrightarrow \text{D} + \text{Mn(II)} \quad (4)$
	$\text{Tyr} + \text{Cl}_2 + \text{MnO}_2 \longrightarrow \text{E} + \text{Mn(II)} \quad (5)$
	$\text{BP-4} + \text{Cl}_2 + \text{MnO}_2 \longrightarrow \text{F} + \text{Mn(II)} \quad (6)$
	$\text{Mn(II)} + \text{Cl}_2 \longrightarrow \text{MnO}_2 \quad (7)$
Intermediate/end products: A, B, C, D, E, F	

Scheme 1. The main reaction pathway of a Tyr and BP-4 mixture during (1) KMnO_4 preoxidation and (2) chlorination.

3.3. The roles of KMnO_4 preoxidation in genotoxicity production

(i) Precursor oxidation by KMnO_4 and MnO_2

The genotoxicity of Tyr, BP-4 and their mixture before/after the KMnO_4 preoxidation was determined. We found that none of them exhibited genotoxicity (Fig. S4). Hence the KMnO_4 preoxidation could not directly affect the genotoxicity production. However, it altered the speciation of precursors and formed DBPs, and thus indirectly affected the genotoxicity production from chlorination.

MnO_2 was formed *in situ* as the reduction product of KMnO_4 , which could also oxidize Tyr (Fig. S5). To determine its oxidation effect on Tyr, we compared the genotoxicity production of Tyr after chlorination with and without 2-h MnO_2 preoxidation (Fig. S6). The result indicates that MnO_2 also can cause the decomposition of Tyr and further affect the genotoxicity production from chlorination, which is consistent with KMnO_4 results (Fig. 1). However, the reduction in genotoxicity with MnO_2 preoxidation was lower than that with KMnO_4 preoxidation under the same dosage ($[\text{KMnO}_4]_0/[\text{MnO}_2]_0$: $[\text{Tyr}]_0 = 1:1$ and $1.5:1$). This can be ascribed to the normal oxidation potential of KMnO_4 ($E^\theta = 1.70$ V) being higher than that of MnO_2 ($E^\theta = 1.22$ V). The intermediates in Tyr oxidation by MnO_2 may produce chlorination byproducts with higher genotoxicity than those produced with KMnO_4 oxidation.

(ii) Oxidation of chlorination byproducts by KMnO_4 and MnO_2

During preoxidation, KMnO_4 oxidation has little effect on the removal of BP-4 (Fig. S3). It can be deduced that the KMnO_4 mainly oxidized the chlorination byproducts of BP-4 to affect the genotoxicity production. To demonstrate this, the role of KMnO_4 oxidation during BP-4 chlorination was investigated. Results indicated that increasing KMnO_4 dosage significantly decreased the genotoxicity of chlorinated BP-4 (Fig. 2(a)), which accounts for the genotoxicity reduction of chlorinated BP-4 with KMnO_4 preoxidation. For Tyr, KMnO_4 was completely consumed during peroxidation (Fig. S1(a)). The MnO_2 present during chlorination included residual MnO_2 from KMnO_4 preoxidation and newly formed MnO_2 from Mn(II) chlorination. Based on the previous literature, the rate constant of the reaction of Tyr with chlorine (10^5) (Na and Olson, 2007; Armesto et al., 1993) was approximately 3–4 orders of magnitude higher than that of Tyr with MnO_2 (10^1 – 10^2) (Altaf et al., 2009). During chlorination, the residual Tyr would rapidly react with HOCl rather than MnO_2 , and then the chlorinated

intermediate products may react with MnO_2 . To investigate the effect of MnO_2 oxidation during Tyr chlorination, we determined the genotoxicity of Tyr after chlorination in the presence of different dosages of MnO_2 . The results indicate that MnO_2 can further oxidize the intermediate products of Tyr to inhibit the genotoxicity production (Fig. 2(b)). Remarkably, the genotoxicity reduction of chlorinated Tyr by MnO_2 reached 14.1%, which only accounted for a third of genotoxicity reduction (47.5%) after KMnO_4 preoxidation (the optimal dosage $[\text{KMnO}_4]_0/[\text{MnO}_2]_0$: $[\text{Tyr}]_0 = 1:1$). We concluded that the genotoxicity reduction for chlorinated Tyr was attributed to the combined impacts of the oxidation of Tyr by KMnO_4 and MnO_2 and the oxidation of chlorination byproducts by MnO_2 .

(iii) Cl_2 consumption competition between Mn(II) and BP-4/intermediates/residual Tyr

Mn(II) can be oxidized by chlorine as previously shown (Allard et al., 2013; Hao et al., 1991). During chlorination, dissolved Mn(II) is initially oxidized to MnO_2 . Thereafter, an autocatalytic process takes place. The Mn(II) is quickly adsorbed on the surface of newly formed MnO_2 and then is oxidized on its surface by HOCl (Hao et al., 1991). Under the same chlorine dosage, organics (e.g., intermediates, residual Tyr and BP-4) would react with less chlorine in the presence of Mn(II), compared to that without Mn(II), thus possibly affecting the genotoxicity production.

To investigate the effect of chlorine dosage on genotoxicity production, we determined the genotoxicity of BP-4 without preoxidation (Fig. 3(a)) and Tyr with (Fig. 3(c,d)) and without (Fig. 3(b)) preoxidation under various dosages of chlorine, respectively. Results revealed that the chlorine dosage has little effect on the genotoxicity of chlorinated BP-4 (Fig. 3(a)). That is to say, the competition of Mn(II) for chlorine could be ignored during BP-4 chlorination. However, the genotoxicity of chlorinated Tyr was significantly decreased with increasing chlorine dosage (Fig. 3(b)). There may be two reasons for this. One is that after reactions with more chlorine, these intermediate chlorinated aromatic DBPs might decompose to more aliphatic ones, leading to lower toxicity (Li et al., 2015; Liu and Zhang, 2014; Yang and Zhang, 2013). The second reason is that more chlorine promotes the transformation from N-DBPs to C-DBPs. Briefly, during chlorination, Tyr could be transformed into two kinds of by-products during chlorination (Chu et al., 2012), i.e., N-DBPs with high genotoxicity and carbonaceous by-products (C-DBPs) with low genotoxicity (Pan et al.,

Fig. 2. The genotoxicity of chlorinated by-products formed from (a) BP-4 (0.1 mM) during KMnO_4 oxidation; (b) Tyr (0.1 mM) during MnO_2 oxidation ($[\text{KMnO}_4]_0/[\text{BP-4}]_0/[\text{MnO}_2]_0$: $[\text{Tyr}]_0 = 1:10, 1:5, 1:2, 1:1$ and $1.5:1$). Error bars are the same as those in Fig. 1 $t = 24$ h, $\text{pH} = 6.0$ and $T = 25 \pm 0.2$ °C.

Fig. 3. Genotoxicity of (a) chlorinated BP-4 (0.1 mM) (b) chlorinated Tyr (0.1 mM) (c) chlorinated Tyr with KMnO_4 preoxidation ($[\text{KMnO}_4]_0:[\text{Tyr}]_0 = 1:1$) and (d) chlorinated Tyr with KMnO_4 preoxidation ($[\text{KMnO}_4]_0:[\text{Tyr}]_0 = 1:5$) under different chlorine dosages ($[\text{Cl}_2]_0:[\text{BP-4}]_0/[\text{Tyr}]_0 = 15:1, 20:1$ and $30:1$). Error bars are the same as those in Fig. 1. Preoxidation conditions: $t = 2$ h, $\text{pH} = 6.0$ and $T = 25 \pm 0.2$ °C. Chlorination conditions: $t = 24$ h, $\text{pH} = 6.0$ and $T = 25 \pm 0.2$ °C.

2016; Muellner et al., 2007; Plewa et al., 2004). On the basis of previous study, N-DBPs from Tyr were first generated and then hydrolyzed to C-DBPs (Chu et al., 2012). It has been proved that N-DBPs were in degradation after 6-h contact time. So under the excessive chlorine condition, increasing chlorine dosage would accelerate the hydrolysis of N-DBPs at 24 h, thus decreasing the genotoxicity. So the competition of Mn(II) for chlorine may significantly affect the genotoxicity production of Tyr during chlorination. After KMnO_4 preoxidation, whether chlorine dosage still affected the genotoxicity production from Tyr was investigated. The results revealed that the chlorine dosage had a slight effect on the genotoxicity of the preoxidation-chlorination products from Tyr when Tyr was completely consumed during preoxidation ($[\text{KMnO}_4]_0:[\text{Tyr}]_0 = 1:1$) (Fig. 3(c)). However, a significant decrease ($p < 0.01$) in genotoxicity was observed with increasing chlorine dosage when the amount of residual Tyr reached 60% (Fig. S1) in the chlorination ($[\text{KMnO}_4]_0:[\text{Tyr}]_0 = 1:5$) (Fig. 3(d)). This indicated that the competition of Mn(II) for chlorine mainly affected the genotoxicity production from the residual Tyr.

To verify the role of Mn(II) oxidation in the genotoxicity production, we measured the genotoxicity of Tyr with different concentrations of Mn(II) ($0\text{--}1.5 \text{ mg L}^{-1}$) after chlorination. The dosed Mn(II) concentrations maintained a level similar to that present after 2-h KMnO_4 preoxidation of Tyr. Results showed that the genotoxicity first increased and then decreased with increasing Mn(II) dosage (Fig. 4). This demonstrates that the competition for chlorine between Mn(II) with a low dose and Tyr could significantly increase the genotoxicity production. This is responsible for the

Fig. 4. The genotoxicity of Tyr (0.1 mM) with different concentrations of Mn(II) ($0.05 \text{ mg L}^{-1}, 0.1 \text{ mg L}^{-1}, 0.4 \text{ mg L}^{-1}$ and mg L^{-1}) after chlorination. Error bars are the same as those in Fig. 1 $t = 24$ h, $\text{pH} = 6.0$ and $T = 25 \pm 0.2$ °C.

increase in genotoxicity production for chlorinated Tyr at $[\text{KMnO}_4]_0:[\text{Tyr}]_0 = 1:10$ and $1:5$ conditions. However, the genotoxicity production was reduced when the Mn(II) dose was increased ($0.8 \text{ mg L}^{-1}\text{--}1.5 \text{ mg L}^{-1}$). This may be due to the formation of more MnO_2 from Mn(II). The oxidation by MnO_2 rather than

the competition effect played a dominant role to decrease the genotoxicity of chlorinated Tyr.

3.4. Comparison of genotoxicity production between the mixture of Tyr and BP-4 and the sum of Tyr and BP-4 individually after the KMnO_4 preoxidation-chlorination process

After KMnO_4 preoxidation and chlorination of Tyr, BP-4, and their mixture, we compared the genotoxicity production for these three precursors to see whether the genotoxicity of the mixture was equal to the sum of individual genotoxicity values. A significantly higher effect on the genotoxicity production of mixtures within the range $[\text{KMnO}_4]_0:[\text{precursor}]_0 = 0-1.5$, and a significantly lower effect within $[\text{KMnO}_4]_0:[\text{precursor}]_0 = 1.2-1.5:1$ than the sum of genotoxicity values from individual precursors were observed after the preoxidation-chlorination process (Fig. 5). This may be due to Tyr and BP-4 interacting after the preoxidation-chlorination process to produce a synergistic or antagonistic effect, thus affecting the genotoxicity production of the chlorinated mixture. To investigate the role of the combined effect, we examined the genotoxicity of the post preoxidation-chlorination mixture (In this situation, Tyr and BP-4 were preoxidized and chlorinated, and then the chlorine was quenched; finally they were mixed) and compared it with the sum of genotoxicity values of Tyr and BP-4 after chlorination with KMnO_4 preoxidation. We found that the post preoxidation-chlorination mixture had a significantly higher genotoxicity (a synergistic effect) at $[\text{KMnO}_4]_0:[\text{mixture}]_0 = 1:10$, $1:5$ and $1:2$ conditions, and a lower genotoxicity (an antagonistic effect) at $[\text{KMnO}_4]_0:[\text{mixture}]_0 = 1:1$ and $1.5:1$ conditions than the sum of individual genotoxicity values ($p < 0.01$) (Fig. S7). This is the main cause for the augmentation and reduction of genotoxicity production from the chlorinated mixture after preoxidation. Notably, after preoxidation, the genotoxicity augmentation for the chlorinated mixture was enhanced at $[\text{KMnO}_4]_0:[\text{mixture}]_0 = 1:10$

and $1:5$ conditions compared to that without preoxidation (Fig. 5, inset). This suggests that only KMnO_4 preoxidation with a proper dose can effectively control the genotoxicity augmentation of the chlorinated mixture. In actual water, the genotoxicity of mixtures after chlorination or chlorination and preoxidation was not estimated from the genotoxicity of the individual precursors.

4. Conclusions

This study investigated the role of KMnO_4 preoxidation on the production of genotoxicity for Tyr/BP-4 individually and in mixture after chlorination. Results revealed that the genotoxicity production for chlorinated BP-4 decreased with increasing KMnO_4 dosage. During preoxidation, KMnO_4 oxidation had no effect on BP-4. After chlorination, KMnO_4 could oxidize the chlorination byproducts of BP-4 to decrease the genotoxicity. Concurrently, the production of genotoxicity from Tyr/mixture chlorination was reduced only by a proper dose of KMnO_4 preoxidation. There existed an optimal molar ratio of KMnO_4 to Tyr/mixture of $1:1$. The roles of KMnO_4 preoxidation in the production of genotoxicity for chlorinated Tyr include: (i) during preoxidation, KMnO_4 and manganese dioxide (MnO_2 , the reduction product of KMnO_4) could oxidize Tyr, thus altering the species of DBPs to decrease the genotoxicity production; (ii) during chlorination, on one hand, the MnO_2 formed from KMnO_4 reduction and Mn(II) oxidation can further oxidize the chlorinated intermediates of Tyr to decrease the genotoxicity production; on the other hand, the competition of Mn(II) oxidation with Tyr/intermediate oxidation can result in less chlorine reacting with organics to induce an increase in genotoxicity. Additionally, the production of genotoxicity for the chlorinated mixture was first augmented and then reduced with increasing KMnO_4 dosage compared with that of individual chlorinated precursors. Only KMnO_4 preoxidation with a proper dose can effectively control the augmentation of genotoxicity for chlorinated mixtures.

This study highlights the importance of the preoxidant dose in controlling the genotoxicity production of DBPs. KMnO_4 preoxidation with a proper dose is an effective way to decrease the genotoxicity of chlorination byproducts. It is expected that this study will provide new insight to clarify the role of preoxidation in the formation of DBPs by using toxicity assessment instead of product analysis.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.chemosphere.2016.08.018>.

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Fig. 5. The genotoxicity of chlorinated mixture ($0.1 \text{ mM } [\text{Tyr}]_0:[\text{BP-4}]_0 = 8:2$) and the sum of genotoxicity of individual chlorinated precursors with KMnO_4 preoxidation ($[\text{KMnO}_4]_0:[\text{precursor}]_0 = 0, 1:10, 1:5, 1:2, 1:1$ and $1.5:1$). Inset shows the genotoxicity difference between mixture and the sum of individual precursors after chlorination with KMnO_4 preoxidation. G_{mixture} and G_{sum} represent the genotoxicity of the chlorinated mixture and the sum of genotoxicity values of individual chlorinated precursors, respectively. Asterisks indicate that the genotoxicity of the chlorinated mixture was significantly different from the sum of genotoxicity values of individual chlorinated precursors after KMnO_4 preoxidation ($p < 0.05$). Error bars are the same as those in Fig. 1. Preoxidation conditions: $t = 2 \text{ h}$, $\text{pH} = 6.0$ and $T = 25 \pm 0.2^\circ \text{C}$. Chlorination conditions: $t = 24 \text{ h}$, $\text{pH} = 6.0$ and $T = 25 \pm 0.2^\circ \text{C}$.

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