Genotoxicity removal of reclaimed water during ozonation

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Abstract: Genotoxicity in wastewater and reclaimed water now is gaining increased attention because of genotoxins’ potential damage to the ecosystem and human health. In the present study, the effect of ozonation on genotoxicity in reclaimed water was investigated. It was found that ozonation decreased the genotoxicity dramatically in tertiary treatment plants. In the further batch ozonation experiment in laboratory, secondary effluent sample used exhibited the genotoxicity of 41.1 ± 4.1 μg4NQO/L. Ozonation with a dose of 10 mgO₃/L completely removed the genotoxicity in secondary effluent. However, after ozonation, the dissolved organic carbon value of the sample didn’t change much but the specific ultraviolet absorbance (SUVA) value dropped sharply. With the help of Fourier transform infrared spectroscopy, ozonation was found to change chemical aliphatic carbon and C-O of the DOM, which might be the reason of the significant decreases of SUVA and genotoxicity.

Keywords: ozonation; genotoxicity; SUVA; Fourier transform infrared spectroscopy

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Introduction

The water resource crisis is becoming a worldwide problem, thus wastewater reclamation is given as a practical resolution (US EPA, 2004). Unfortunately, it was reported that there are still amount of complex organic pollutants in reclaimed water. Some organic pollutants are toxic or precursors of toxic byproducts, which induced the risk during further use of reclaimed water (Shon et al., 2006; Richardson et al., 2007). Therefore, the safety assessment of reclaimed water is of great importance.

Generally, with the help of chemical and instrumental analysis, we can monitor some
regulated pollutants in reclaimed water, but the toxicity and potential impacts of the pollutants on organisms are still hard to know. As an essential supplement, bioassays can be used to measure toxic effects and assess the safety of reclaimed water containing regulated and unknown contaminants (Shiraishi et al., 2001; Watsona et al., 2012).

Among different types of toxic effects, genotoxicity in drinking water and reclaimed water is drawing researchers’ attention in recent years. It was reported that the reclaimed water from wastewater treatment plants and the surface water affected by reclaimed water often exhibited genotoxicity at a level of $10^{-2} \mu g4NQO/L$ (Aguayo et al., 2004; Escher et al., 2008; Wu et al., 2010; Zhang et al., 2011). Moreover, chlorination was found to decrease the genotoxicity of the reclaimed water when there was a low ammonia nitrogen concentration in it (Wu et al., 2010), but to increase the genotoxicity both in drinking water and reclaimed water with high ammonia nitrogen concentration (Richardson et al., 2007; Wang et al., 2007; Wu et al., 2010).

Since there is the potential risk in wastewater, the application of tertiary treatment is always taken into consideration. Particularly, ozonation as an effective tertiary process is widely used in reclamation treatment plants. As is known to all, ozone as a strong oxidant can easily react with the unsaturated chemical bonds in organics. Also, ozonation was reported to convert the large molecule into smaller ones and raise the ratio of the hydrophilic organic fractions in reclaimed water (Gong et al., 2008).

Moreover, bioassays were also applied to access the effect of ozonation on biotoxicity of reclaimed water (Cao et al., 2009b; Misik et al., 2011; Wei et al., 2012). For example, ozonation was reported to help reduce micropollutants such as endocrine disrupting chemicals and pharmaceutical and personal care products in reclaimed water (Westerhoff et al., 2005; Zhang et al., 2006; Hollender et al., 2009), which may decrease the biotoxicity. Misik et al. (2011) showed that genotoxicity was reduced after ozonation, but further systematic study such as the impact of different ozone doses on genotoxicity is still needed. On contrary, formation of high toxic disinfection byproducts such as bromo-tri-halo methanes, haloacetic acids and nitrosodimethylamine were promoted by ozonation (Andrzejewski et al., 2008; Hollender et al., 2009). The increase of toxic disinfection byproducts formation resulted in negative impacts to ecological environment and human beings.

Among the present studies, although ozonation was reported to be helpful to remove genotoxicity, the relationship between the changes of DOM and the genotoxicity during ozonation and the removal mechanism of the genotoxicity are still unknown. Therefore, the aim of this study is to investigate the changes of the genotoxicity and DOM during ozonation.
and try to find out why the genotoxicity is changed.

1 Materials and methods

1.1 Sampling

Influent samples before ozonation in three tertiary treatment plants (A, B, and C) and sample-Q from secondary effluent of a municipal wastewater treatment plant using anoxic-anaerobic-oxic as a main treatment process were collected respectively. After collection, all the samples were kept with ice and immediately delivered to laboratory. Then they were filtered through 0.45 μm glass fiber filters to eliminate suspended solids, and then adjusted to pH 2.0 with H₂SO₄ and stored at 4 °C. The dissolved organic carbon (DOC) of filtered sample was measured using a Shimadzu TOC-V CPH analyzer and the UV absorbance was measured using a Shimadzu UV-2401PC UV-VIS recording spectrophotometer.

1.2 Ozonation experiment

A batch ozonation reactor was established in laboratory as shown in Fig. 1. It is consisted of an ozone generator, ozone detectors, glass columns, flow meters and dehydrators, etc. Ultra pure oxygen was used as the feeding gas for the ozone generator, and secondary effluent were reacted with the aerated ozone in the glass columns. Both of the input and the exhaust ozone gas was dehydrated before going through ozone detectors to avoid the damage from steams to ozone detectors.

Four liters of secondary effluent was reacting with ozone in each batch. The contacting time of 5 min was applied, and the ozone doses were 0--20 mg/L. After the ozonation, ozone generator was turn off and the ultra pure oxygen was continually aerated into the column for another 10 min to drive the residual ozone.

1.3 Genotoxicity assay

By using Oasis HLB resin cartridges (Waters Corporation, America), organic pollutants in water samples were concentrated according to previous method (Wu et al., 2010). The cartridges were activated by passing 10 mL of methanol and 10 mL of Milli-Q ultrapure water through them. Afterwards, water samples (500 mL) were acidified to pH 2 and passed through the cartridges for concentration, and the cartridges containing adsorbed samples were then dried under a flow of air. Adsorbed organics on each cartridge were eluted with acetone (10 mL) to obtain the extract of the sample. Thereafter, the extract was dried completely under a nitrogen flow and then dissolved in dimethylsulfoxide (DMSO) to obtain 1-200-fold
concentration (volume of sample/volume of extract) for genotoxicity assay.

The genotoxicity of the concentrated samples was evaluated with the SOS/umu test based on *Salmonella typhimurium* TA1535/pSK1002 without S9 activation in triplicates according to ISO 13829. In the experiment, the *Salmonella typhimurium* (200 μL) stored under -80°C was firstly unfreezed and cultivated in medium (10 mL) under 37°C for 12 hr. After one day cultivation, the suspension solution (1 mL) was transferred to 9 mL media and cultivated under 37°C for 1.5 hr. All the steps above were operated in a sterile environment.

Afterwards, the bacteria were further used for contact culture. 4-Nitroquinoline-N-oxide (4NQO) diluted with DMSO solution was applied as positive control. Also, concentrated samples were used for contact culture together with the bacteria. After a three-step cultivation in 96-cell plates, OD595 and OD405 were measure in order and the relative β-galactosidase activity was calculated according to ISO 13829.

The relative β-galactosidase activity of different concentrations of 4NQO was used to plot the dose-response curve of 4NQO as shown in **Fig. 2**. It should be noted that when the relative β-galactosidase activity was below 1, it can be inferred that there was no significant genotoxicity induced by the sample compared to negative control. Therefore, the dose-response curve was a linear fit curve with a fixed intercept at the point (0, 1) and the slope value (4.60) of the curve was obtained.

Similarly, the linear dose-response curve of each sample and the slope of it were also obtained. Then the ratio of the slope value of each sample to that of 4NQO was calculated to represent the genotoxicity of each sample in a 4NQO equivalence form.

Differences in genotoxicity between different samples were considered statistically significant when *p* < 0.05 according to Student’s *t*-test.

### 1.4 Fourier transform infrared spectroscopy

Water samples (500 mL) with different ozone dose (0, 5, 20 mg/L) were freezed dried and the powdered samples were further used for Fourier transform infrared (FT-IR) analysis by a Perkin-Elmer Spectrum GX FTIR spectrometer.

### 2 Results and discussion

#### 2.1 Removal of genotoxicity by ozonation

Firstly, the genotoxicity of reclaimed water samples from tertiary treatment plants A, B and C was studied. The dose-response curves of the three samples before and after ozonation are shown in **Fig. 3**, and the genotoxicity of them are listed in **Table. 1**. After statistical
analysis using Student’s t-test, the genotoxicity in reclaimed water was remarkably removed ($p < 0.05$) with the removal efficiency of 79%-96% after ozonation in all the three tertiary treatment plants. The genotoxicity in the effluent of the ozonation reactors was all at a low value (0.2-5.7 μg 4NQO/L).

**Table 1** Genotoxicity before and after ozonation in the tertiary treatment plants

<table>
<thead>
<tr>
<th>No.</th>
<th>Tertiary treatment process</th>
<th>Before ozonation</th>
<th>After ozonation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Membrane filtration-ozonation-chlorine disinfection</td>
<td>27.8 ± 4.8</td>
<td>5.7 ± 0.2</td>
</tr>
<tr>
<td>B</td>
<td>Sand filtration-ozonation</td>
<td>11.1 ± 1.1</td>
<td>0.5 ± 0.1</td>
</tr>
<tr>
<td>C</td>
<td>Aerobic filtration-ozonation-chlorine disinfection</td>
<td>5.1 ± 0.5</td>
<td>0.2 ± 0.1</td>
</tr>
</tbody>
</table>

*Standard deviation was given for the genotoxicity of each sample (n = 3). Student’s t-test was applied to compare the genotoxicity before and after ozonation for each sample.

Afterwards, ozone at different doses was added into sample-Q in laboratory and then the genotoxicity after ozonation was also monitored (Fig. 4). The genotoxicity in sample-Q before ozonation was 41.1 ± 4.1 μg 4NQO/L which was at a common level for secondary effluent according to previous research (Aguayo et al., 2004; Escher et al., 2008; Wu et al., 2010; Zhang et al., 2011). With the increasing doses of ozone, ozonation significantly removed the genotoxicity ($p < 0.05$). What’s more, the genotoxicity was even below the limit of detection (0.1 μg 4NQO/L) when the dose of ozone was above 10 mg/L. Ozone, therefore, showed a good performance in removing the genotoxicity during tertiary treatment process. In recent research, it was reported that ozone can reduce the genotoxicity (Cao et al., 2009a; Misik et al., 2011; Wei et al., 2012), and the removal curve in this study was even consistent with Wei et al. (2012) and Cao et al. (2009a).

However, it should be noted that in a latest study by Wei et al. (2012), it was found in a reclamation plant that when the genotoxicity of the influents was lower than 10 μg 4NQO/L, a practical ozone dosage which was rather lower than laboratory dosage was not efficient to remove genotoxicity. Besides, Petala et al. (2008) also suggested that ozone doses played an important role in influencing the mutagenic effect and excessive doses may lead to the formation of toxic byproducts.

After all, when comparing all the results, it can be suggested that ozonation under a proper condition is helpful to remove genotoxicity in wastewater and reclaimed water.
2.2 Changes of DOC and SUVA value during ozonation

DOC of sample-Q was measured during ozonation (Fig. 5a). Before ozonation, the DOC of sample-Q was 6.1 ± 0.1 mg/L. After ozonation, the DOC was reduced only a little. Similar results also supported that ozonation decreased DOC or COD quite limitedly (Gong et al., 2008; Petala et al., 2008).

The changes of specific ultraviolet absorbance (SUVA) value (UV$_{254}$/DOC) were also observed during ozonation (Fig. 5b). Unlike the changes of DOC, SUVA was significantly reduced from 1.88 ± 0.1 to 0.71 ± 0.1 L/(mg·m) during ozonation, indicating that there may be a destruction in unsaturated chemical structures. In addition, there was also a similarity between the removal of genotoxicity and SUVA according to the results in Figs. 4 and 5b.

According to previous studies, limited reduction of DOC but dramatic decrease in SUVA was used as an evidence for the destruction of the unsaturated bonds by ozonation (Morrison and Boyd, 1983; Westerhoff et al., 1999). Particularly, aromatic structures of DOM contributed much in SUVA and aromatic compounds such as humic acids, some polycyclic aromatic hydrocarbon and steroids, and some aromatic soluble microbial products are often detected in wastewater and reclaimed water (Barker and Stuckey, 1999). It can be estimated that the SUVA drop may partly result from the degradation of those aromatic compounds.

Besides, Westerhoff et al. (1999) and Zhang et al. (2012) proved that some aromatic toxic chemicals in wastewater and reclaimed water were degraded by ozonation, which indicated that there may be some correlations between the removal of genotoxicity and SUVA/unsaturated carbon.

2.3 FT-IR spectra of the secondary effluent during ozonation

With the help of FT-IR analysis, the changes of functional groups in DOM were further investigated. Normalized FT-IR spectra of sample-Q during ozonation are shown in Fig. 6. Peaks at 3410, 1645, 1493, 1423, 1385, 1141, 1121, 864, 826 and 624 cm$^{-1}$ were observed. Moreover, the absorbance peaks at the wave number of 1300--900 and 1500--1300 cm$^{-1}$ were assigned to C--H bond in aliphatic structures and C--O bond according to literature review (Skoog and Leary, 1992; Stuart, 2005) and the peaks were much more changed rather than the others after ozonation. Particularly, the absorbance at 1121 cm$^{-1}$ increased from 0.74 to 0.80 after ozonation with an ozone dose of 10 mg/L, which indicated an increase of C--O bonds after ozonation. Similarly, an significant absorbance increase (from 0.91 to 0.98) at 1423 cm$^{-1}$ was also found, which suggested that there was an increase of aliphatic structures. Both the two hints above together indicated that the unsaturated structures were broken up during ozonation. This deduction during ozonation was also supported by previous studies (Morrison...
and Boyd, 1983; Westerhof et al., 1999). Based on the results, the genotoxicity and SUVA were both reduced and the FT-IR results supported destruction of aromatic structures, it can be suggested that the removal of the genotoxicity was possibly related to the changes of the aromatic DOM during ozonation.

3 Conclusions

Ozonation significantly removed the genotoxicity of the secondary effluent. During ozonation, DOC value was changed little but SUVA value dropped remarkably. FT-IR analysis revealed that C-H bond in aliphatic structures and C-O bond were increased after ozonation. It indicated that the unsaturated bonds in the DOM, especially those in the aromatic structures, did react with the ozone, which resulted in the decreases of the SUVA and may be related to the genotoxicity drop of secondary effluent. Therefore, those unsaturated carbon in wastewater and reclaimed water, especially aromatic chemicals/structures, should be paid more attention to for their potential as toxicity indicators during reclamation.

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References


Protection Agency: Washington, DC.


Fig. 1 Schematic diagram of laboratory ozonation system.

Fig. 2 Linear dose-response curve of 4NQO. Triplicate measurements were conducted and the slope value of the linear curve was 4.60.
Fig. 3 Linear dose-response curves of sample A, B and C before and after ozonation. Triplicate measurements were conducted and the slope values of the linear curves of A, B, C, A+ozonation, B+ozonation and C+ozonation were 0.128, 0.0511, 0.0234, 0.0261, 0.0022 and 0.0010, respectively.

Fig. 4 Removal of genotoxicity during ozonation (sample-Q). Error bars represent the standard deviation ($n = 3$). The symbol “#” means the genotoxicity is significant decreased ($p < 0.05$) according to Student’s $t$-test and the symbol “-” means the genotoxicity is below the limit of detection (0.1 μg4NQO/L).
Fig. 5 Variation of DOC (a) and SUVA (b) changes during ozonation (sample-Q). Error bars represent the standard deviation ($n = 3$).
Fig. 6 FT-IR spectra of the secondary effluent during ozonation (sample-Q).