Evaluation of drinking water treatment combined filter backwash water recycling technology based on comet and micronucleus assay

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ABSTRACT

Based on the fact that recycling of combined filter backwash water (CFBW) directly to drinking water treatment plants (WTP) is considered to be a feasible method to enhance pollutant removal efficiency, we were motivated to evaluate the genotoxicity of water samples from two pilot-scale drinking water treatment systems, one with recycling of combined backwash water, the other one with a conventional process. An integrated approach of the comet and micronucleus (MN) assays was used with zebrafish (Danio rerio) to investigate the water genotoxicity in this study. The total organic carbon (TOC), dissolved organic carbon (DOC), and trihalomethane formation potential (THMFP), of the recycling process were lower than that of the conventional process. All the results showed that there was no statistically significant difference (P > 0.05) between the conventional and recycling processes, and indicated that the genotoxicity of water samples from the recycling process did not accumulate in 15 day continuous recycling trial. It was worth noting that there was correlation between the concentrations of TOC, DOC, UV254, and THMFPs in water and the DNA damage score, with corresponding \( R^2 \) values of 0.68, 0.63, 0.28, and 0.64. Nevertheless, both DNA strand breaks and MN frequency of all water samples after disinfection were higher than that of water samples from the two treatment units, which meant that the disinfection by-products (DBPs) formed by disinfection could increase the DNA damage. Both the comet and MN tests suggest that the recycling process did not increase the genotoxicity risk, compared to the traditional process.

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Introduction

For low turbidity water, the removal ability for particulates is weaker during the traditional coagulation process compared to high turbidity water, due to the relatively slow hydrolysis of coagulant, stronger water viscosity and slower settling velocity of flocs (Xiao et al., 2009). Consequently, the corresponding chemical stability of the effluent could be reduced dramatically. Based on this phenomenon, recycling of the combined filter backwash water from WTP was proposed as a...
novel method to improve the traditional treatment technology for treating low turbidity water. Some previous studies explored the removal efficiency of organics, Giardia cysts and Cryptosporidium oocysts, and some routinely determined parameters to evaluate the water quality safety after recycling of sludge (Cornwell and Lee, 1994a; Cornwell et al., 1987; Walsh and Gagnon, 2006). Gottfried et al. (Gottfried et al., 2008) stated that the addition of reused backwash water solids was beneficial in increasing the collision and adhesion probabilities of suspended particles to further enhance the traditional treatment technology, and notably higher removal efficiency for DOC and UV$_{254}$ was found, when the raw water blended with 5% and 10% by volume of filter backwash water was re-input into the conventional drinking water treatment. Xu et al. (2009) and Zhou et al. (2012) thought that the remaining amorphous aluminum and ferric hydroxide in the waste sludge probably did not fully react during the traditional process, and thus could be used as aggregated cores in the recycling process, enhancing the collision probabilities among particles. According to other studies, the removal of Cryptosporidium oocysts could be enhanced by 4.3%–20% when untreated filter backwash water was recycled to the input of the coagulation process (Cornwell and Lee, 1994; Cornwell et al., 1987; Cristale et al., 2013). As mentioned above, recycling CFBW is a feasible practice, and there may be optimum operating conditions and water quality ranges with regard to the recycling process, which can not only improve the coagulation efficiency for treating low turbidity water, but also save water resources and the cost of coagulants. However, some unknown toxic substances may accumulate in the recycling process, and these contaminants may influence the effluent quality, which could pose a potential threat to human health on conditions of long-time exposure, owing to waste residuals from CFBW raw water pollution that can be produced during drinking water treatment (Buschini et al., 2008). Furthermore, that may be interactive effects among the components, although the individual physico-chemical parameters meet the water quality guidelines (Routledge et al., 1998). Therefore, toxicity evaluation of the recycling process is a useful tool to determine the comprehensive risk. To evaluate the toxicity of water samples treated by the recycling process and compare the toxicity of water from two pilot-scale WTP, comet and micronucleus (MN) assays have proved to be highly sensitive means to detect DNA damaged by a mixture of pollutants (Andrighetti-Fröhner et al., 2006; Biscardi et al., 2003). In fact, genotoxicity has also been measured directly in treated water using in vivo tests with fish, newts, Vicia faba, Allium cepa and so on (Monarca et al., 2004). The comet assay can detect primary DNA lesions (i.e., single or double strand breaks) by measuring the migration of DNA fragments from immobilized nuclear DNA (Singh et al., 1988). The MN assay has been extensively used to study the clastogenic effects as micronuclei derived from chromosome breakages, which in fish and freshwater mussel gill cells have demonstrated high sensitivity for monitoring surface water and detecting the genotoxicity of drinking water (Minissi et al., 1998). Some studies have appeared that employed the comet and MN assays on zebra fish to evaluate the removal efficiency of genotoxicity in the anoxic-oxic process, showing a high incidence of MN frequency when the peripheral erythrocytes of fish were exposed to pollutants (Zhang et al., 2013). In addition, the MN frequency is associated with cancer prediction (Bolt et al., 2011).

This study was focused on application of a combined bioassay and chemical analysis approach to evaluate the potential genotoxicity and water quality risk of water samples treated by the recycling process in comparison with water samples treated by a conventional process. Based on this, a pilot WTP was constructed to conduct 15-day continuous recycling trials, and the genotoxicity of different water samples was assessed by comet and MN assays of zebra fish.

1. Materials and methods

1.1. Pilot-scale experimental setup and physico-chemical analysis

1.1.1. Pilot-scale experimental setup and procedure

A sketch of the pilot treatment processes is shown in Fig. 1, illustrating the conventional and recycling drinking water treatment processes, respectively. The design parameters of the recycling process units were the same as for the conventional process. The influent flow rate was 5 m$^3$/hr. The A unit contains two parts: one is a grid flocculation tank, with a bottom length of 1100 mm, a bottom width of 400 mm and a liquid height of 1700 mm, the other is a plate sedimentation tank, with a bottom length of 2100 mm, a bottom width of 800 mm and a height of 1600 mm. The plate component is composed of 63 plates with a tilt angle of 60° and interval of 20 mm. The B unit is a rapid filter tank with filtration velocity of 8 m/hr. The recycled sludge was stored in two custom-built sludge storage tanks, with a diameter of 1500 mm and a height of 1500 mm. The sludge was pumped to the head of the static pipeline mixer after being completely mixed. The whole process cycle time was 44 min. The WTP waste residual was collected in the grid flocculation tank (label A in Fig. 1) and rapid sand filter (label B in Fig. 1) of the recycling process every 24 hr, and then the waste residual was released to the sludge storage tank (label E in Fig. 1) and filter backwash water tank (label D in Fig. 1), respectively, through a diameter 100 mm PVC pipe.

The water treatment plant (WTP) waste residuals were recycled from tanks D and E to the head of the static mixer by a peristaltic pump via a rubber hose of diameter 10 mm and ensured that the residual could be completely reused under the optimal treatment combination. With this method, this pilot plant test was continuously operated for 15 days and the WTP waste residual could be repeatedly used many times until the determined parameters exceeded the sanitary standards for drinking water of China. To test the water quality stability of the recycling process and compare it with the conventional process, the turbidity, TOC, DOC, UV$_{254}$, SUVA and THMFPs of water from different sampling points (label C1, C2, C3, R1, R2, R3 and R4 in Fig. 1) were determined every day. However, for genotoxicity evaluation, the day 5, day 10, and day 15 were chosen as sampling times.

1.1.2. Coagulant and characterization of drinking water treatment plant (WTP) waste residuals

The polyferric aluminum chloride (PFAQ) used was industrial grade (with content of 8.1% Fe$_2$O$_3$ and 3.3% Al$_2$O$_3$, basicity...
6.2%, Zibo, China). The source water originated from the Nenjiang River was obtained from the intake of Zhongyin water treatment plant (Daqing, China). The water was characterized as typical slightly polluted surface water with low turbidity and color. The WTP waste residuals were from the sedimentation tank sludge and filter backwash water. The sludge employed in this study was taken from the outlet of the Grid flocculation tank, and was seriously contaminated compared to the raw water samples, with high TOC and turbidity. The filter backwash water was essentially a low-solids wastewater with organics. The detailed characteristics of raw water and sludge are shown in Table 1.

1.1.3. Physico-chemical analysis
Turbidity was measured with a turbidimeter (HACH2100P, Hach Company, USA) according to US EPA method #180.1. DOC and TOC were analyzed by a TOC analyzer (TOC-V, Shimadzu Corporation, Japan). The UV absorbance at 254 nm (UV254) was determined using a spectrometer (DR5000 UV/VIS).

Fig. 1 – Diagram of the pilot-scale drinking water treatment plant. A: grid flocculation tank; B: conventional rapid sand filter; C: clean-water reservoir; D: filter backwash water tank; E: sludge storage tank; F: dosing pump; C1: effluent of flocculation tank in conventional process; C2: effluent of rapid sand filter in conventional process; C3: effluent after post-disinfection with chlorine dioxide in conventional process; R1: effluent of flocculation tank in recycling process; R2: effluent of rapid sand filter in recycling process; R3: effluent after post-disinfection with chlorine dioxide in recycling process; R4: blended water originated from raw water, sludge and filter backwash water; P1: backwash water originated from rapid sand filter; P2: sludge originated from Grid flocculation tank.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Raw water</th>
<th>Sedimentation tank sludge</th>
<th>Filter backwash water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turbidity (NTU)</td>
<td>4–7</td>
<td>500–2560</td>
<td>250–460</td>
</tr>
<tr>
<td>Color (CJ)</td>
<td>28–35</td>
<td>350–420</td>
<td>85–160</td>
</tr>
<tr>
<td>COD$_{M_n}$ (mg/L)</td>
<td>5–9</td>
<td>18–35</td>
<td>10–21</td>
</tr>
<tr>
<td>UV$_{254}$ (cm$^{-1}$)</td>
<td>0.065–0.076</td>
<td>0.091–0.298</td>
<td>0.072–0.081</td>
</tr>
<tr>
<td>TOC (mg/L)</td>
<td>4.4–5.6</td>
<td>4.85–7.2</td>
<td>4.61–5.32</td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>3.85–4.65</td>
<td>4.70–6.01</td>
<td>4.53–5.16</td>
</tr>
<tr>
<td>Solid content (w%/w%)</td>
<td>0.001–0.006</td>
<td>1.02–3.2</td>
<td>0.02–0.98</td>
</tr>
<tr>
<td>pH</td>
<td>6.1–8.3</td>
<td>6.1–7.9</td>
<td>6.3–8.1</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>3–10</td>
<td>4–12</td>
<td>4–11</td>
</tr>
<tr>
<td>Trihalomethanes (THM$_4$) (μg/L)</td>
<td>80–159</td>
<td>142–207</td>
<td>132–221</td>
</tr>
<tr>
<td>THMFPs (μg/L)</td>
<td>395–557</td>
<td>438–567</td>
<td>456–630</td>
</tr>
</tbody>
</table>
Hach Company, USA). Both DOC and UV254 were measured after filtration through 0.45 μm membranes. The solid content of the recycled sludge was examined following the Standard Methods (APHA, 1995) and the pH was measured by a pH meter (PHS-3C, Leici Company, China). The THMFP was also determined, as the parameter estimates the expected concentration of THMs in water samples of conventional and recycling processes with an excess of free chlorine (APHA, 1998).

1.2. Toxicity assay

1.2.1. Genotoxicity assay

The comet and micronucleus assays were employed to assess the genotoxicity of the conventional and recycling process water samples. Zebrafish were obtained from a local fish market and kept in glass aquaria at 24 ± 2°C with constant aeration and 48 h water changes. The feeding environment had a 12:12 (light:dark) photoperiod and the dissolved oxygen in water was above 2 mg/L. Fish were fed with commercial fish food daily. Ten juvenile zebrafish (0.39 ± 0.11 g) were placed in 3.5 L glass jars, and each treatment was replicated three times; the zebrafish of both sexes (3–5 month[s] old) were exposed for 2 months to test the genotoxicity. The negative control (NC) and positive control (PC) groups were maintained in dechlorinated tap water and potassium dichromate (0.02 mg/L) solution, respectively.

1.2.1.1. Comet assay. Single cell gel electrophoresis (SCGE) was employed to detect the DNA strand breaks caused by the chemical material gene toxicity. Blood samples were obtained by puncture of the peripheral erythrocytes of fish, and immediately injected into a micro centrifuge tube together with 10 μL heparin sodium and phosphate buffered saline (PBS), and then centrifuged at 2000 r/min for 10 min and used to obtain a cell suspension (about 106 cells/mL). Cell viability ≥ 87% was ensured by evaluation with trypsin blue. Rough microscope slides were coated with two layers of agarose. For the first layer, 0.8% normal melting point (NMP) agarose was spread over the slides and solidified on ice. The cell suspension was immobilized in 0.7% low melting point (LMP) agarose at a ratio of 1 part cell suspension to three parts LMP agarose and solidified on ice. Afterwards the cell suspension immobilized in microgel was subjected to incubation in 100 mmol/L EDTA, 2.5 M NaCl, 1% Triton X-100 and 10% DMSO (pH 13.0) for 1.5 hr in the dark at 4°C. Then the microgel was submerged in an electrophoretic buffer (300 mmol/L NaOH, 1 mmol/L EDTA at pH > 13) for 20 min to unwind the DNA at 4°C. Finally the microgel was placed into an electrophoresis chamber containing an electrophoresis buffer. After electrophoresis in the same buffer at 25 V and 300 mA for 20 min, samples were neutralized by incubation in 400 mM Tris at pH 7.4 for 2 min. After neutralization (0.4 mol/L Tris–HCl, pH 7.5), the slides were stained with 100 μL ethidium bromide (10 μL/mL) and observed at a magnification of 320× using a fluorescence microscope (BX51/TF, Olympus Company, Japan) equipped with an excitation filter of 518 nm and an image-analysis system with a grey-scale CCD camera and Comet 3.0 software (Kinetic Images, Liverpool, UK). For each test, the tail moments of 100 randomly selected cells were analyzed. Tail moment (tail length multiplied by the fraction of DNA in the tail) was used as the measure of DNA damage (Peycheva et al., 2014; Singh et al., 1988).

1.2.1.2. Micronucleus assay. For the MN assay, the zebrafish peripheral blood sample was dropped onto clean slides containing fetal bovine serum and dried. Then the blood cells were fixed in methanol for 20 min and dried at room temperature. Afterwards the slides were stained with Giemsa solution (Nanjing Jiancheng, China) in phosphate buffer solution (PBS, pH 6.8) for 15 min, then washed with PBS, and dried at room temperature before microscopic analysis. The MN frequency was determined to evaluate the genotoxicity (Al-Sabti and Metcalfe, 1995; Arkhipchuk and Garanko, 2005).

2. Results and discussion

2.1. Water sample quality analysis

Both TOC and DOC are important surrogate parameters that can represent the content of organic matter in drinking water treatment. As is well known, organics are potential threats to human health and are difficult to remove by the conventional treatment process. Chlorine is the most extensively used disinfectant in China, and disinfection by-products (DBPs) is produced when the chlorine reacts with organics, such as humic and fulvic acids. With the purpose of evaluating the coagulation performance of the recycling process and investigating the variation of DBP concentration in water samples collected from each treatment unit, the THMFPs were determined. In addition, the traditional parameters TOC and DOC were also monitored during the whole process. The water quality parameters of the different treatment units are illustrated in Fig. 2. It can be seen that the average removal efficiencies of TOC, DOC, and THMFPs between the recycling process and conventional processes in the coagulation/flocculation units are 34.8% and 29.3%, 25.7% and 21.5%, and 18.9% and 17.2%, respectively. The mean concentrations of TOC (3.79 mg/L), DOC (3.42 mg/L), and THMFPs (362.3 μg/L) in the filter units of the recycling process were less than those of the conventional process, where the corresponding TOC, DOC, and THMFP values are 3.91 m, 3.65, and 385.7 μg/L, respectively. Compared with the effluent from the rapid sand filter, there was no significant removal of TOC, DOC and THMFPs after disinfection in the two WTPs. The results appeared to show that the sludge recycling process did not exacerbate the water quality; in contrast to the conventional process, the removal efficiency for organic matters was clearly improved.

2.2. Genotoxicity assays

2.2.1. Comet assay

The comet assay is widely used to detect DNA damage, which includes single-strand breaks, double-strand breaks, and incomplete excision repair sites (Shi et al., 2009). To a certain extent, comet assays can represent the presence of genotoxic compounds in water. The water samples from every stage of the conventional and recycling process were evaluated by comet assay (tail moment), and the results are demonstrated...
in Fig. 3. All the water samples showed significant differences compared with the NC. The effluent genotoxicity of the grid flocculation unit (C1, R1) was not remarkably reduced in comparison with raw water from the two drinking water treatments during the 15 day continuous trials. However, the effluent genotoxicity of effluent from the conventional rapid sand filter (C2, R2) was approximately 2–3 times lower than that of raw water. The results are probably due to the fact that a large number of particles coated with natural organic matter (NOM) were present in the flocculation effluent, hence the NOM was removed from water along with particles in the filter unit.

Considering the tail moment, C1–C3 and R1–R3 correspond to the conventional and recycling processes, respectively. Clearly, there were no significant differences (P > 0.5) for the three sampling times, suggesting that genotoxic compounds did not accumulate during the recycling process. It was worth noting that the genotoxicity of C3, R3, and R4 was higher than for other sample points. After blending raw water, filter backwash water and sludge, a great quantity of pollutants was imported in the recycling process, resulting in higher content of genotoxic compounds in R4.

Table 2 shows the degree of DNA damage in zebra fish peripheral blood, according to tail intensity, which was classified into five levels of 0, 1, 2, 3, or 4 (from undamaged 0 to maximally damaged 4), arbitrary units (AU) were used to express the extent of DNA damage and were calculated as shown below (Zhong et al., 2001).

\[
AU = \sum_{i=0}^{4} Ni \times i
\]

where, Ni is the number of cells with degree i, i is the damage degree (0, 1, 2, 3, 4).

Fig. 2 – Concentration of TOC, DOC, and THMFPs in water sample treated by recycling and conventional process during 15 day continuous trials. C1–C3 and R1–R3 refer to the caption of Fig. 1.

Fig. 3 – Comet assay on zebra fish of drinking water sampled at different points of plants between conventional and recycling process. Statistical analysis was performed using Student’s t-test after ANOVA for the Tail moment. Data are presented as mean ± SD (n = 3). * P < 0.05 compared with NC; the same letter (i.e., a–i) at P > 0.05 compared with the counterpart of treatment units at different sampling times.
All water samples from different sampling points could induce DNA damage, and a statistically significant difference (P < 0.05) was observed compared with the NC. The DNA damage scores decreased as the zebra fish were exposed to the effluent of the grid flocculation tank and conventional rapid sand filter, independent of treatment process and sampling time. However, the DNA damage scores dramatically increased after disinfection (P < 0.05) compared with the counterpart of treatment units at different sampling time. The same letters represent no obvious difference between these two kinds of pilot-scale Water Treatment Works. Therefore, there was similar genotoxicity between C1 and R1, C2 and R2, and C3 and R3 at three different sampling times.

To investigate the relationship between the concentrations of TOC, DOC, UV254, THMFPs and the DNA damage score, correlation analysis was performed. As demonstrated in Fig. 4, linear regression goodness-of-fit values (R2) between the DNA damage score and TOC, DOC, UV254, and THMFPs were 0.68, 0.63, 0.28 and 0.06, respectively. In general, as the measured organics concentration increased, the DNA damage score increased as well. However, there was poor correlation between UV254 and the DNA damage score. The value of UV254 only represents a type of organic matter having 254 nm wavelength ultraviolet absorbance, like humic natural macromolecular organic matter and certain aromatic compounds (including C=C double bonds and C=O double bonds), but cannot reflect the total content of organic matter in water. For every treatment unit, the change trend of DNA damage score was consistent in the comet assay.

### 2.2.2. Micronucleus (MN) assay

The water samples collected from every unit of the recycling and conventional process were evaluated by MN assay, and results are shown in Fig. 5. The MN frequency of all water samples was significantly higher than that of NC, which indicated that both the raw water and treated water may contain genotoxic pollutants. For the three sampling times, MN frequency did not change in C1 and R1, C2 and R2, and C3 and R3. In addition, the MN frequency steadily declined during the flocculation and filter processes in both of the treatments. Yet, the values of MN frequency (C3 and R3) were even much higher after disinfection, which strongly indicated that some genotoxic DBPs were generated. These genotoxic compounds still pose potential threat of DNA strand breakage, chromosome breakage or chromosome loss, although they could not be detected by TOC or CODMn measurements due to their trace quantities in water.

Most importantly, the MN frequency was not enriched in the recycling process over the 15 day continuous recycling trial.

### 3. Discussion

TOC, UV254, and DOC, as important organic pollution parameters, can indicate the level of chemicals that sometimes result from raw water pollution or are produced during water treatments. Fig. 4 illustrates that the concentration of TOC and DNA damage score exhibited positive correlation (R2 = 0.68),
which meant that the NOM in water probably posed potential danger to human health. However, the values of the conventional monitoring parameters in Fig. 2 and values of genotoxicity in Figs. 2–4 of water samples produced by the recycling process were not obviously increased, which forcefully indicated that the recycling process could be used as an alternative method to treat low turbidity water, saving water resources. In addition, Zhou et al. (2012) stated that the removal of organic substances by hydrolyzed metal coagulants is likely to cause a neutralizing effect. The anionic sites over the surface of organic materials could be bound by metal species like Al\(^{3+}\) and Fe\(^{3+}\) present in the PFAC, and then particles formed by this means can be removed during the subsequent sedimentation or filtration units. We speculate that some remaining metal composition could also be present in the discharged sludge, and produce metal-organic complex precipitation again via the charge neutralization mechanism during the recycling process. In addition, the adsorption of organic substances on amorphous metal hydroxide precipitates also played a significant role in the removal of DOC and UV\(_{254}\) (Sharp et al., 2006). The sludge, with its porous nature and large specific surface area, can strongly adsorb soluble organic materials, which meant that recycled sludge could be used as a good adsorbent for removing organic substances from water. The enhancement of organics removal in this study still mainly resulted from the joint effects of complexation and adsorption by the insoluble aluminum hydroxides. Gottfried et al. (2008) also found that raw water blended with 5% and 10% by volume of filter backwash water showed significantly higher removal efficiency of DOC. With the filter backwash water recycled in the raw water, the destabilized
particles could appreciably improve the amount of collision sites with the soluble NOM constituents in the raw water. This procedure may most likely affect the formation of flocs during the coagulation and sedimentation stages (Cornwell and Lee, 1994). An analysis of the mechanism of organic materials removal is shown in Fig. 6.

McCormick et al. (2010) investigated the disinfection by-product (DBP) concentration and formation potential in filter backwash water (FBWW), and evaluated the impact of untreated FBWW recycle on water quality in conventional drinking water treatment. They stated that the particulate organic material contained within FBWW is available for reaction with chlorine to form DBPs; however, blending of untreated 10% FBWW with raw water ahead of the rapid mixing stage of the plant’s treatment train did not impact DBP concentrations. In our results, there was no obvious difference in the THMFP concentration in water samples between these two treatment processes, showing that there was no impact on the effluent treated by the recycling process in terms of DBPs. Our results were in accordance with the previous research. In addition, the only slightly higher concentrations of TOC, DOC and UV254 in the sedimentation tank sludge and filter backwash water in all cases (in Table 1), illustrated that there was the possibility that organic materials could be released again in water samples after the waste sludge was reused. That is the reason that the genotoxicity of R4 was significantly higher than other water samples in these two water treatments. Both the comet assay and MN test are considered sensitive techniques for evaluating genetic damage, which reflect different genetic endpoints in two different cell populations. The Comet assay is used to detect primary DNA lesions (i.e., single or double strand breaks), while the MN assay is used to detect structural and numerical chromosomal damage. Additionally, the comet assay determines strand breaks and labile sites that are subsequently removed by repair enzymes (Heuser et al., 2008). In this study, both of the detecting techniques were successfully applied in detecting DNA damage in the two different drinking water treatment processes. The results of the two genotoxicity tests indicated that there was no statistically significant difference ($P > 0.05$) between the conventional and recycling processes (Figs. 3 and 5, Table 2), and the genotoxic materials were not accumulated during the recycling process over the 15 day continuous recycling trial.

Furthermore, the results of the comet and MN assay suggested that the disinfection procedure with liquid chlorine led to the increase of DNA damage for C3 and R3 at three sampling times. Fig. 3 also shows that there was a higher concentration of THMFPs in raw water, which has great potential to produce chloroform after addition of the liquid chlorine to water. These DBPs formed by disinfection could enhance DNA damage. Moreover, other reports have shown that DNA strand breaks and MN frequency could increase after disinfection, while cell viability would decrease with changes in oxidative stress potential. (Shi et al., 2009). Many DBP compounds are produced in chlorination, and isolated DBPs (i.e., bromo-organic by-products and halonitromethanes) were found to induce DNA damage in Salmonella or in mammalian tests (Richardson et al., 2007). For disinfection of raw water by peracetic acid, chlorine dioxide or sodium hypochloride, the comet assay test on both haemolymph of zebra mussels and human white blood cells showed dramatic seasonal variations in DNA damage capability (Bolognesi et al., 2004; Laffon et al., 2001).

4. Conclusions

The results of this study clearly showed that the recycling process could greatly improve the coagulation efficiency, including organic material removal. The water quality of water samples treated by the recycling process did not deteriorate in
comparison with the conventional process. There were certain relationships between the concentrations of TOC, DOC, UV254, THMFPs in water and the DNA damage score, with $R^2$ values of 0.68, 0.63, 0.28, and 0.64, respectively. All the water samples exhibited significantly higher tail moment and MN frequency than the NC, which indicated that both the raw water and treated water may contain genotoxic pollutants. There was no significant difference ($P > 0.5$) in the sampling points C1 and R1, C2 and R2, and C3 and R3 in tail moment and MN frequency at the three sampling times. In addition, the MN frequency steadily declined during the flocculation and filter processes in both of the treatments. Yet, the values of MN frequency (C3 and R3) were much higher after disinfection, which strongly indicated that some genotoxic DBPs were generated. The genotoxicity of water samples from the recycling process did not accumulate in the 15 day continuous recycling trial, with both comet and MN assays showing similar results.

Acknowledgments

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