Advanced treatment of landfill leachate membrane concentrates: performance comparison, biosafety and toxic residue analysis

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ABSTRACT

With the improvement of people's consciousness about health, more attention has been paid to the biosafety of effluent reaching conventional discharge standard. In this contribution, removal efficiency of COD, acute toxicity, genotoxicity and estrogenicity in landfill leachate membrane concentrates (MCs) among UV-Fenton, Fenton and activated carbon adsorption process were compared. *D. magna* acute toxicity assay, comet assay, cytokinesis-block micronucleus and E-screen assay were performed to assess whether the effluent reaching the main parameters of Chinese Discharge Standard (GB 16889-2008) still had toxic residues. Under the conditions that COD of effluents treated by three processes were up to the discharge standard, no obvious toxic residue was found in the effluent of UV-Fenton treatment, but effluent from Fenton or activated carbon adsorption process showed genotoxicity or estrogenicity to some extent. Dynamic analysis of UV-Fenton degradation process for estrogen simulation solutions was also conducted, and the formation of intermediates was detected by GC/MS. Toxic residues might be caused by the lack of treatment duration and the formation of more toxic intermediates. UV-Fenton was found to be efficient for the treatment of MCs. Biosafety should be concerned when new wastewater discharge standard being established.

Key words | advanced treatment, biosafety of effluent, landfill leachate, membrane concentrates, toxic residue analysis

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INTRODUCTION

The widespread use of membrane-based treatment processes for landfill leachate disposal creates an excess of landfill leachate membrane concentrates (MCs). With higher concentration of refractory and toxic organics than raw landfill leachate, MCs have a huge threat to environment and human health. These abundant refractory pollutants include long-chain hydrocarbons, halohydrocarbons, aromatic compounds, humic and fulvic acids, and chlorinated organics (Zhang *et al.* 2013).

Due to the high salinity and refractory organic content, MCs have a low biodegradability and the contribution of biological treatment to MCs treatment is limited (Singh & Tang 2013; Qin & Chen 2016). Advanced oxidation processes (AOPs) which can generate strong oxidizing hydroxyl radicals (•HO) are promising methods for MCs treatment (Méndez *et al.* 2015). AOPs have been demonstrated to be a feasible solution to treat landfill leachate for their efficient and non-selective oxidation (Kattel *et al.* 2016; Zha *et al.* 2016). Activated carbon with large amount of micropores can adsorb dissolved contaminants efficiently. Due to its high efficiency, relatively low cost and renewable ability, activated carbon has been successfully used in wastewater treatment (Li *et al.* 2010; Margot *et al.* 2013). Hybrid process of AOPs-activated carbon adsorption for MCs treatment in China has been investigated in pilot scale in Fenggang, Dongguan, Tianziling and Hangzhou landfills. Effluents treated by this hybrid process can reach a favorable effect.

Classical wastewater treatment plants are not built to remove organic micropollutants, resulting in the detection of micropollutants in the environment (Rozas *et al.* 2016). These organic micropollutants include pharmaceuticals and personal care products (PPCPs), pesticides, phthalates and artificial sweeteners, etc. (Mailler *et al.* 2015). These micropollutants have a link to estrogenic, mutagenic or

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genotoxic effects of aquatic organisms, leading to serious biosafety risk (Richard *et al.* 2014). Biosafety assessments of municipal wastewater secondary effluent were previously reported (Freitas *et al.* 2017; Sun *et al.* 2017). It indicates that effluents discharged from traditional wastewater treatment plants are not completely biosafe especially when influents contain more and more new synthetic compounds. The composition of MCs is more complex than common wastewater treatment plants effluents, so MCs treatment effluents deserve more attention about biosafety. In the previous study (Wang *et al.* 2016a; Wang *et al.* 2016b), acute toxicity, genotoxicity and estrogenicity of MCs were determined. But the biosafety of MCs which treated up to the main parameters of discharge standard (GB 16889-2008) such as COD and NH_4-N still have no concrete research.

Here, studying a nanofiltration MC, we directly compared the efficiency of three different advanced treatments, Fenton, UV-Fenton, and activated carbon adsorption, on toxicity reduction (via analytical chemistry, acute toxic activity of *Daphnia magna*, estrogenic toxic effects of MCF-7 cells and genotoxicity effects of HepG2 cells). Moreover, toxicity evaluation of the effluents treated by the three advanced treatment methods and meeting main parameters of Chinese discharge standard were investigated. The degradation of PAEs (typical EDCs) in the nanofiltration MC was used to analyze the remaining toxicity.

METHODS

Samples of MCs and sample disposal

In this research, the MCs were sampled in a landfill in Longgang, Shenzhen, China, and the landfill has been run for more than 14 years and about 1,000–1,500 m^3 of leachates were disposed per day. After biological treatment, nanofiltration treatment is used for further purification. And the MCs are formed during the nanofiltration treatment. MCs were sampled weekly in April 2016. MCs were homogenized by mingling into glass containers. Prior to analyze, MCs were stored in a refrigerator at 4 °C. After the collection of untreated and treated MCs, physical and chemical parameters were determined immediately.

Advanced treatment experiments of MCs

Two-liter containers with aeration equipment and ultraviolet light were used to conduct UV-Fenton process. The experiment comprised pH adjustment (pH:2-3), addition of solid iron sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$, 2 g/L) and hydrogen peroxide solution (H_2O_2, 4% v/v). At every time points, samples were moved from the photoreactors into the flask beakers for test or store until analysis at 4 °C. NaOH solution (50%, w/w) was used to adjust the pH to 8–9 for terminating the reaction and precipitating residual irons. All experimental procedures of Fenton treatment were the same except ultraviolet irradiation used in the UV-Fenton treatment. Sequential batch reactors were used for activated carbon adsorption treatment. The dosage of activated carbon was 1.5 g/L. The adsorption treatment proceeded in oscillation (200 rpm) at the temperature of 20 °C. At every time intervals, activated carbon was separated from the solution, before further moved into flask beakers for test or store until analysis at 4 °C.

D. magna acute toxicity assays

D. magna acute toxicity assays were conducted referred to US Environment Protection Agency (EPAUS 2002). Five concentrations (6.25, 12.5, 25, 50, and 100%) of untreated and treated MCs were chosen. The EC_{50} was used to represent the level of acute toxicity.

Genotoxicity tests

HepG2 cells for genotoxicity tests were bought from a biochemistry laboratory in the Jinan University First Affiliated Hospital and maintained in Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum at 37 °C and 5% CO_2.

Cytokinesis-block micronucleus (CBMN) assays were performed referring to a previous publication (Wang *et al.* 2016a). Samples preparation of untreated and treated MCs referred to the publication, mainly about extraction of genotoxic active ingredients from MCs. Plates with 6 wells were used to maintain HepG2 cells (2×10^5 cell/well) for 24 h. HepG2 cells in exponential growth period were used to conduct exposure experiments with different concentrations of treated and untreated MCs. In all experiments, positive (mitomycin C, 0.3 μg/mL) controls and negative (only culture medium) controls were conducted. The values of micronucleus and cytokinesis-block proliferation index (CBPI) were used to assess the level of genotoxicity. The CBPI were calculated according to the equation: $CBPI = (M1 + 2M2 + 3Mn)/N$, and $M1$, $M2$, Mn represented the number of cells with one, two, multi nuclei, respectively. N represented the total number of cells scored.

The comet assays (alkaline single-cell gel electrophoresis) were conducted referring to an earlier study (Azqueta & Collins 2013) with small changes (30 min electrophoresis at 25 V). Plates with 6 wells were used to maintain HepG2 cells (1×10^5 cell/well) for 24 h. HepG2 cells in exponential growth period were used to conduct exposure experiments with different concentrations of treated and untreated MCs. In all experiments, positive (mitomycin C, 0.3 μg/mL) controls and negative (only culture medium) controls were conducted. The percentage of DNA (% DNA in tail) was used to assess the level of DNA damage.

E-screen tests

Sample preparation of untreated and treated MCs for genotoxicity test was referring to a previous publication (Gong *et al.* 2014), mainly about extraction of estrogenic active ingredients from MCs.

MCF-7 cells for genotoxicity tests were bought from a biochemistry laboratory in the Jinan University First Affiliated Hospital and maintained in Dulbecco's Modified Eagle's Medium (DMEM) without phenol red, but supplemented with 10% non-hormone fetal bovine serum and 1% penicillin/streptomycin (1,000 U/ml penicillin and 1,000 U/ml streptomycin) at a humidified atmosphere of 37 °C and 5% CO_2.

The E-screen tests were performed referring to an earlier study (Gadd *et al.* 2010). After the extraction process above of untreated and treated MCs, dimethylsulfoxide (DMSO) was added as a solvent replacement to get exposure solution. After culturing in a 96-well plate (104 cells/well) for 24 hours, non-hormone MCF-7 cells were washed twice with PBS, and then they were exposed to several concentrations of exposure medium for 48 hours. Internal positive controls, negative controls without hormones and solvent controls (DMSO) were conducted to all assays. Each well was added 20 μL (5 mg/L) of MTT after 48 hours and continued to be cultured for 4–6 hours until infected media were discarded. DMSO solution of 150 μL was added to each well, before vibrating for 10 min at a low speed in the darkness to dissolve completely. Optical density (OD) of each well at the wavelength of 490 nm was detected by automatic microplate reader (Multiskan MK3, Thermo Fisher, USA). The proliferation effect (PE) was used to assess the level of estrogenicity and calculated with the following formula:

$$PE = \text{the OD of experimental groups} / \text{the ODof negative controls}$$

Dynamics and intermediate analysis

Preparation of estrogen simulation solutions (ESS) is as follows. Dimethyl ortho-phthalate (DMP, CAS 131-11-3), Di-n-butyl ortho-phthalate (DBP, CAS 84-74-2) and Bis (2-ethylhexyl) ortho-phthalate (DEHP, CAS 117-81-7) were purchased from A ChemTek, Inc. (Worcester, USA). DMP of 0.1000 g, DBP of 0.1000 g or DEHP of 0.1000 g was put into a 100-mL volumetric flask respectively to prepare a 1,000-mg/L single standard stock solution with methanol and stored in the dark at 4 °C before use. One milliliter of each three single standard stock solutions above was transferred to a 10-mL volumetric flask separately and filled to volume with methanol to prepare single standard stock solutions of 100 mg/L which were stored at 4 °C before use. The single standard stock solution of 2 mL (100 mg/L) was added to 1-L ultrapure water to prepare single standard water sample (DMP of 200 μg/L, DBP of 200 μg/L, DEHP of 200 μg/L) and mixed standard water sample (DMP of 200 μg/L, DBP of 200 μg/L and DEHP of 200 μg/L). The mixed standard samples were used as ESS. Single standard samples and mixed standard samples were treated with the same UV-Fenton process for MCs. The chemical pathways of PAEs changing in ESS were analyzed by GC/MS with some adjustments based on references (Kuch *et al.* 2010; Li *et al.* 2014) during the UV-Fenton process. At different setting time of 0, 5, 10, 15, 30, 60 min, 80-ml samples were removed from the photoreactor and divided into two parts. Sodium chloride of 3 g was added to 50-ml sample to prevent its emulsification and extracted three times with 20-ml dichloromethane by liquid-liquid extraction in a 150-ml separatory funnel. The organic phase was collected, dried by anhydrous sodium sulfate and evaporated to dry using a rotary evaporator. Finally the product was diluted with hexane to 1 ml.

RESULTS AND DISCUSSION

Characterization of untreated MCs

Considering the climatic conditions of sampling points, characteristics of treatment technology and long-term monitoring of water quality, for comprehensive detection of refractory and toxic organics, April with high and stable COD values was chosen as sampling times. The physical and chemical characterization of untreated MCs were listed in Table 1. Untreated MCs, brown liquid, with very low value of $BOD_5/COD = 0.055$, which lead to a low biodegradability. It had a nearly neutral pH and contained

Table 1 | Characterization of MCs

Parameter	COD_{cr} (mg/L)	BOD_5	NH_4-N (mg/L)	Conductivity(ms/cm)	Cl^-(mg/L)	pH	SS(mg/L)
Concentrations	724	43	11.3	11.3	2,925.7	7.5	128

high concentration of Cl-1 (2925.7 mg/L), COD_{cr} (724 mg/L) and conductivity (11.3 ms/cm).

The removal efficiency of COD_{cr} after UV-Fenton, Fenton and activated carbon adsorption treatment

Figure 1 showed the removal efficiency of COD_{cr} after UV-Fenton, Fenton and activated carbon adsorption processes. After 120-min Fenton and UV-Fenton process, the COD_{cr} of concentrate decreased to 69.1% and 80.1% respectively. UV-Fenton had a better removal efficiency of COD_{cr} than Fenton treatment because UV radiation could enhance the formation of hydroxyl free radicals, which could oxidize almost all organic compounds non-selectively (Hu *et al.* 2011; Liu *et al.* 2014). Activated carbon adsorption treatment showed a favorable COD_{cr} removal efficiency of 76.8%, but the removal efficiency showed no significant difference after 20 min because of the adsorption saturation.

Acute toxicity assay results of untreated and treated MCs

D. magna toxicity test results were present in Table 2. The calculation of EC_{50} value was based on the sigmoidal concentration-response curves fitted by the least-squares methods (Ribé *et al.* 2012). Untreated MCs showed acute toxic effect to *D. magna* with EC_{50} value of 15.04%. The high toxicity might be caused by complex components in MCs. Synergistic effects should be considered which made an important contribution for leachate toxicity (Chen *et al.* 2015). Concentrates treated with UV-Fenton at time point of 30 min showed an obvious toxicity reduction ($EC_{50} = 23.5\%$, $p<0.05$). On the contrary, the acute toxic effect significantly increased ($EC_{50} = 7.28\%$, $p<0.01$) after 30-min Fenton process. This might be due to the Fenton oxidation of complex organic contaminants did not result in a fast mineralization, with formation of carbon dioxide and inorganic species, but more poisonous oxidation intermediate products formed. Both Fenton and UV-Fenton treatment effluents showed no acute toxic effect to *D. magna* after 120 min. Concentrates treated with activated carbon adsorption at time points 10, 20, 30, and 40 min showed respective EC_{50} of 27.4, 36.4, 36.8 and 40.7%. Although activated carbon adsorption treatment showed an excellent toxicity reduction to *D. magna*, the toxicity reduction effect did not change obviously after 40 min.

Genotoxicity assay results of HepG2 cells exposed to treated and untreated MCs (micronucleus assays and comet assays)

Micronucleus assay results of HepG2 cells exposed to treated and untreated MCs

Figure 2 showed the mean value of CBPI and MN resulting from HepG2 cells exposed to treated and untreated MCs. Figure 2(a) clearly showed that untreated MCs could result

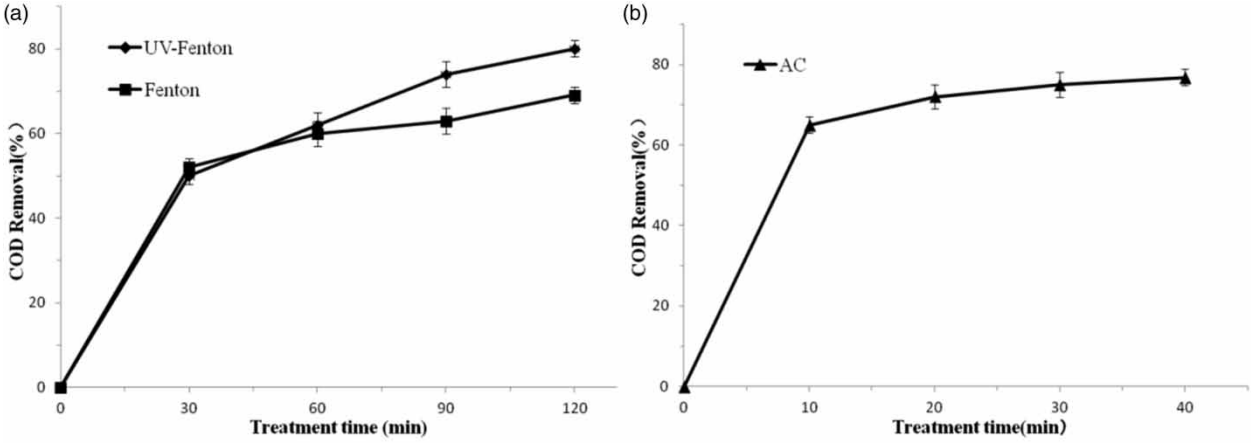

Figure 1 | Removal rate of COD after UV-Fenton, Fenton or activated carbon adsorption treatment; AC: activated carbon.

Table 2 | EC_{50} values of *D. magna* exposed to treated MCs

	EC_{50}%		
Treatment time(min)	**UV-Fenton**	**Fenton**	**AC**
0	15.04	15.04	15.04
10	–	–	27.4
20	–	–	36.4
30	23.5	7.28	36.8
40	–	–	–
60	24.6	16.7	40.7
90	24.2	21.4	40.7
120	25.1	26.3	40.8

AC: activated carbon.

in an obvious induction of the appearance of MN, even at the diluted concentration of 5% ($p < 0.05$). Moreover, with the increase of untreated MCs concentration, the number of MN increased. According to the above-mentioned facts, untreated MCs clearly had genotoxic and cytotoxic potency. The genotoxicity of MCs determined in our study was consistent with landfill leachate investigated in other researches (Toufexi *et al.* 2013; Ghosh *et al.* 2014). High concentration of refractory organics (chromaticity, COD) might cause the genotoxicity (Gajski *et al.* 2012). Compared with negative controls, UV-Fenton treatment effluents of different concentrations showed no obvious difference in the number of MN. On the other hand, Fenton treatment effluents of different concentration showed slight increase of MN compared with negative controls. By contrast, samples treated by activated carbon adsorption still had an obvious genotoxicity and at the diluted concentration of 20%, the numbers of micronucleus showed a significant difference compare with negative control ($p<0.05$). With the concentration of activated carbon adsorption treatment effluent increasing, the numbers of micronucleus increased. Furthermore, samples treated by three methods showed no significant difference in CBPI compared to negative controls. In conclusion, UV-Fenton, Fenton and active activated carbon adsorption treatment all could reduce the genotoxicity to some extent, but UV-Fenton treatment effluents showed no genotoxic effect indicating that UV-Fenton process had a highest removal efficiency of genotoxic matters. Meanwhile, the Fenton and activated carbon adsorption treatment effluents after 120 min still showed some genotoxic effects because the formation rate of

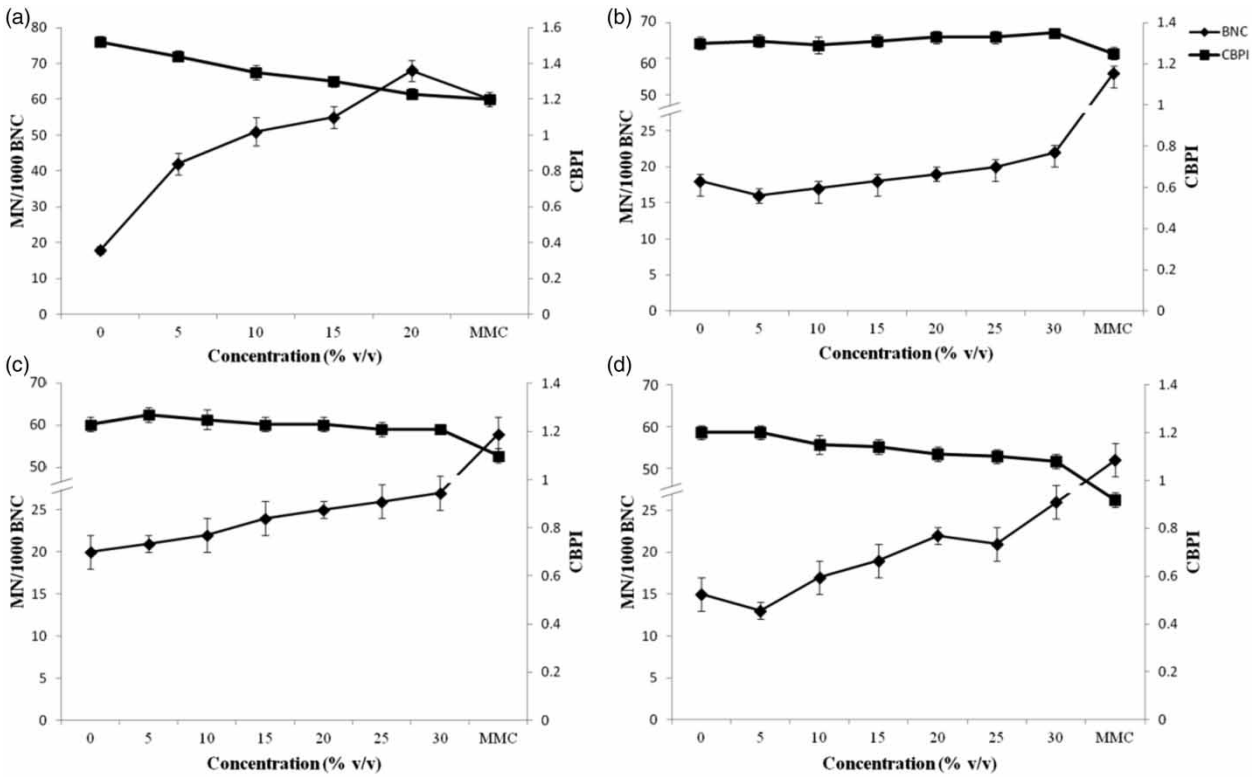

Figure 2 | Micronucleus assay results of HepG2 cells exposed to treated and untreated MCs for 24 h; MMC: Mitomycin C (0.3 μg/mL), BNC: binucleated cells, MN: micronuclei, and CBPI: cytokinesis block proliferation index. (a) Untreated, (b) UV-Fenton treated, (c) Fenton treated and (D) Activated carbon treated.

hydroxyl free radicals in Fenton process was relatively slow and the activated carbon adsorption was nonselective and easy to be adsorption saturated.

Comet assay results of HepG2 cells exposed to treated and untreated MCs

Figure 3 showed the value of %DNA in tails resulting from HepG2 cells exposed to treated and untreated MCs. Compared with negative controls (DNA in tail of 1.25 ± 0.10%), untreated concentrates with the lowest concentrations lead to an obvious increase in DNA in tails (10.49 ± 0.44%). % DNA in tails of HepG2 cells increased with the increase of untreated MCs concentration (Figure 3(a)). MCs treated by Fenton, UV-Fenton or activated carbon adsorption showed reduction of %DNA in tail to some degree compared with untreated MCs. But the %DNA in tail of UV-Fenton treatment effluents showed no obvious difference compared with negative controls (Figure 3(b)). %DNA in tail of Fenton reagent treatment effluents showed slightly increase compared with negative control (Figure 3(c)). And 11.85 ± 2.53% of DNA in tail was determined at the highest concentration of 30%. Activated carbon adsorption treatment effluent showed a higher DNA damage effect compared to Fenton reagent treatment effluents (Figure 3(d)). HepG2 cells exposed to different concentrations of activated carbon treatment effluents showed a significant dose-response DNA damage. The comet assay results were consistent with micronucleus assay results, both assay results showed that the untreated MCs had genotoxicity and the removal efficiency of genotoxicity followed the order of UV-Fenton> Fenton > activated carbon adsorption. Furthermore, among three treatment methods, only UV-Fenton treatment effluent showed no obvious genotoxicity in these two assays even though the parameters of later two treatment effluents such as COD and NH_4-N were up to the Chinese Discharge Standard (GB16889-2008).

Estrogenicity assay results of MCF-7 cells exposed to treated and untreated MCs

The PE of untreated and treated MCs liquid extracts were showed in Figure 4. Untreated MCs showed a significant PE and with the increase of dilution multiple, PE increased gradually until reaching the maximum of 140% at dilution ratio of 135 times, and then began to decrease. With the concentration of MCs decreased, the inhibition effect weakened while the PE value increased, further reduction of concentration resulted in the decrease of PE due to the reduction of estrogenic matters in MCs. After 60-min

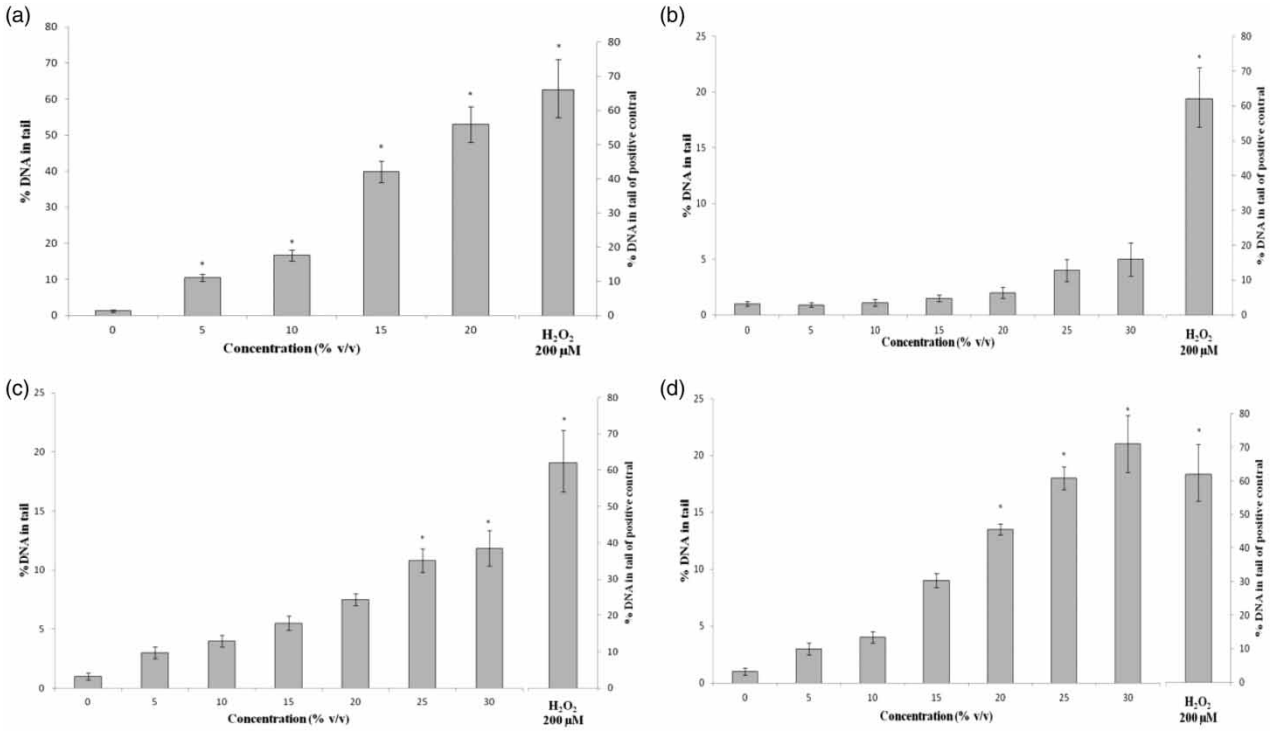

Figure 3 | Comet assay results of HepG2 cells exposed to treated and untreated MCs for 24 h; (a) Untreated, (b) UV-Fenton treated, (c) Fenton treated and (d) Activated carbon treated.

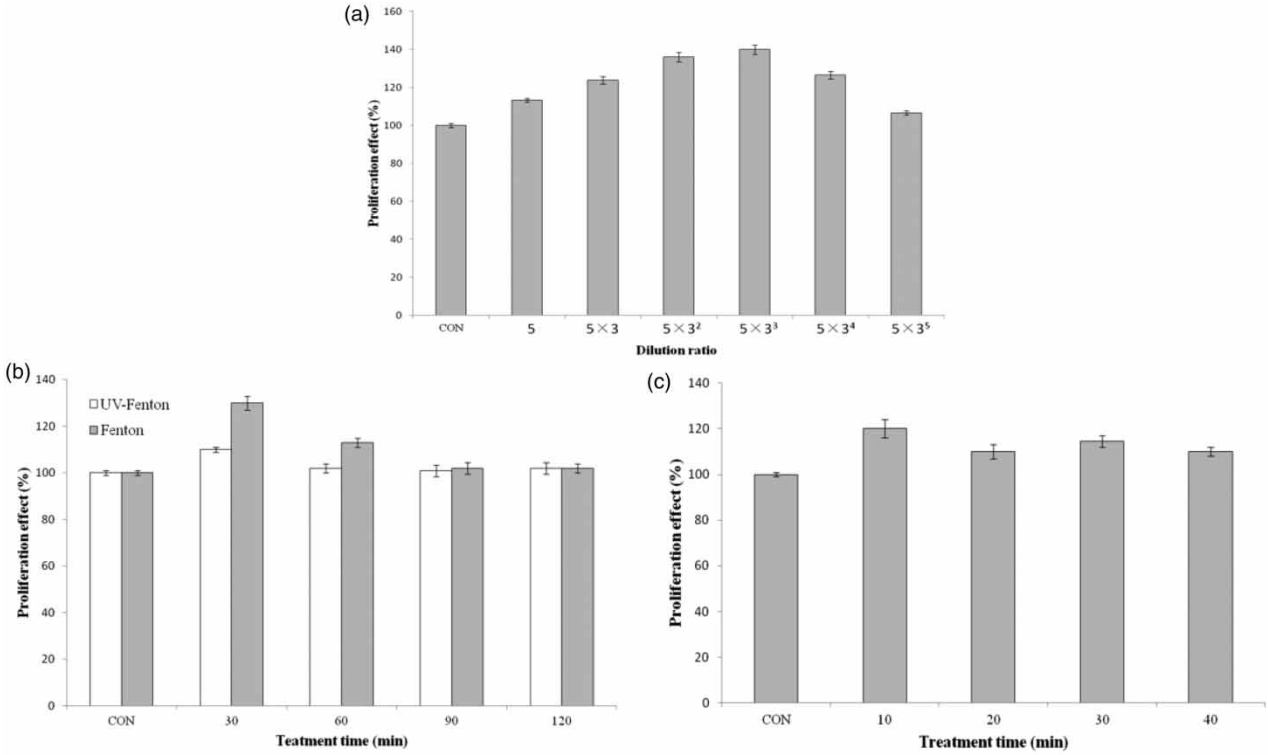

Figure 4 | E-screen assay results of MCF-7 cells exposed to treated and untreated MCs for 48 h; (a) Untreated, (b) Fenton treated and UV-Fenton treated, and (c) Activated carbon treated; CON: Control.

UV-Fenton or 90-min Fenton treatment, the PE of effluents showed no significant difference compared with negative control. By contrast, the PE value of final activated carbon adsorption treatment effluent was 110% meant an estrogenicity residue. Considering the problem of regeneration of activated carbon and secondary pollution, and relatively inefficient Fenton treatment, UV-Fenton was an efficient and promising method for reduction of estrogenicity in MCs.

Dynamics analysis of UV-Fenton oxidation process

It was proved that the untreated MCs had an obviously estrogenicity in above experiment which indicated that there were EDCs in untreated MCs. So common EDCs, including DMP, DBP and DEHP (all were common PAEs), detected in landfill leachate which had estrogenic effects and could cause health problems in humans and animals (Kuch *et al.* 2010), were selected to conduct the simulation of EDCs UV-Fenton AOP. In fact, it had been proved that the hydroxyl free radicals generated during the AOPs could be used to degrade PAEs (Garcia-Segura *et al.* 2013; Li *et al.* 2016). The simulation of the correlation between ln (C_0/C_t) and UV-Fenton treatment duration were given in Figure 5. The ultimate degradation efficiencies of DMP, DBP and DEHP were 98.7%, 93.6% and 89.4% respectively. The linear correlation coefficients r^2 of the DMP, DBP and DEHP were 0.953, 0.976 and 0.962, respectively, which indicated that there was a significantly linear relationship between ln (C_0/C_t) of the three PAEs and the UV-Fenton treatment duration. In short, it was in accordance with the first-order kinetics model. The dynamics analysis results indicated that the lack of treatment duration might lead to the remaining of PAEs in MCs and further result in estrogenicity.

Analysis of intermediates derived from the EES UV-Fenton degradation process

The total ion chromatogram of ESS prior to and after UV-Fenton process for 10 min with GC/MS analysis was given in Figure 6. Since peak areas of 1, 2 and 3 representing the DMP, DBP and DEHP respectively had decreased obviously, it was proved that all the three PAEs could be degraded by the UV-Fenton process to some extent. The peaks 4–13 represented the formation of intermediates in the degradation process. The peak 5 (Retention time: 7.85 min, m/z: 222 149) and peak 6 (Retention time:

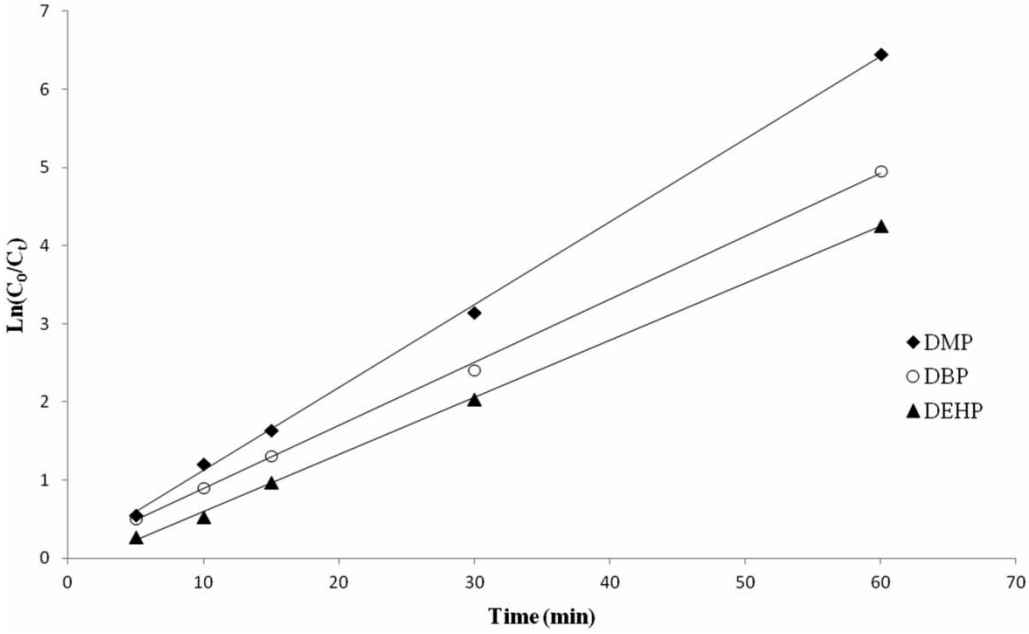

Figure 5 | Degradation kinetic fitting curves of different phthalic acid esters during UV-Fenton process; C_0: concentration at time point 0 min, C_t: concentration at time point t min.

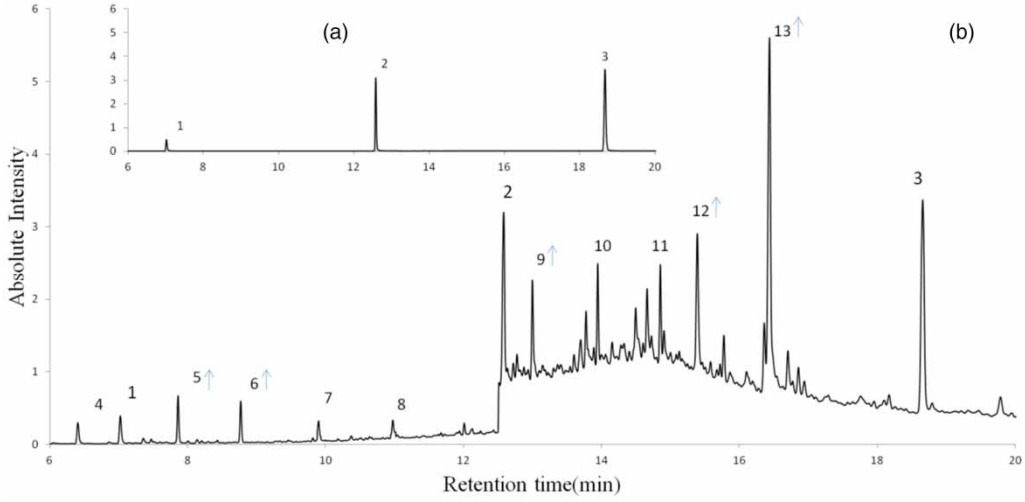

Figure 6 | Intermediates analysis results tested by total ion current gas chromatogram on GC/MS; (a) Untreated, (b) Treated by UV-Fenton process for 10 min; Peak1, 2 and 3 represented DMP, DBP, DEHP respectively. Peak 4–13 represented other compounds formed in the UV-Fenton process. The arrows represented peak areas increasing significantly compared with that UV-Fenton treated for 5 min.

8.71, m/z: 149 205) were determined to be Mono-n-butyl phthalate and DEP, respectively. The previous studies showed that hydroxyl free radical generating from AOPs gave priority to attack the side chain of DBP and formed Mono-n-butyl phthalate and DEP (Wu *et al.* 2015). And the DMP was detected in the single DBP degradation process. Since the retention times of peaks 9, 12 and 13 were longer than peak 2 and shorter than peak 3, the structures of the substances presented by the peaks 9, 12 and 13 were more complex than the peak 2 of DBP and simpler than the peak 3 of DEHP. Therefore, we inferred that the three substances were 2-ethylhexyl-n-butyl phthalate, diisophenyl ortho-phthalate and benzyl-n-butyl ortho-phthalate respectively. Due to no matched structures in the standard mass spectrum library and more complex chemical structures, other intermediates couldn't be confirmed. The

degradation of PAEs in the ESS started from the side chains and split into various phthalate monoesters, ultimately oxidized into small-molecule substances such as carbon dioxide and water. The generation of intermediates during UV-Fenton oxidation process might be one of the reasons causing the estrogenicity residue. Because these intermediates might be more refractory and have a higher estrogenicity.

CONCLUSIONS

After UV-Fenton, Fenton and activated carbon adsorption treatment, the COD_{cr} of effluents were 144, 224 and 168 mg/L respectively. Diluted effluents tested in toxicity assessment assays were up to the Chinese Discharge Standard (GB16889-2008). Effluent after 120-min UV-Fenton treatment showed no obvious toxicity compared to negative control. By contrast, effluents treated by Fenton or activated carbon adsorption treatment showed genotoxicity or estrogenicity to some extent. Therefore, MCs treated effluents which reached the physicochemical discharge standard, still had a biosafety risk. Because of better oxidation depth of refractory organics, UV-Fenton treatment effluents show no obvious toxicity compared with Fenton treatment effluents. Due to the non-selected adsorption and adsorption saturation, the toxicity removal efficiency of activated carbon adsorption generally could not get a satisfactory level and the regeneration of activated carbon still was a problem considering the cost. Dynamics and intermediates analysis of PAEs degraded by UV-Fenton process showed that UV-Fenton is a promising MCs treatment technology, but partial oxidation and the production of intermediates might lead to toxicity residue. Though, we only analyzed the oxidation process of PAEs representing estrogenicity, but similar results could be obtained for other toxic organics (Méndez *et al.* 2015; Zhu *et al.* 2016).

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