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 chemical oxygen demand (COD), acute toxicity, genotoxicity and estrogenicity in landfill leachate membrane concentrates (MCs) among UV-Fenton, Fenton and activated carbon adsorption process were compared. Daphnia 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 the 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 gas chromatography–mass spectrometry (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 a new wastewater discharge standard is being established.

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 are a huge threat to the 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. 2016).

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). A hybrid process of AOPs-activated carbon adsorption for MCs treatment in China has been investigated at 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
genotoxic effects on aquatic organisms, leading to a serious biosafety risk (Richard et al. 2014). Biosafety assessments of municipal wastewater secondary effluent have been 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 regarding biosafety. In the previous study (Wang et al. 2016a; Wang et al. 2016b), the acute toxicity, genotoxicity and estrogenicity of MCs were determined. But the biosafety of MCs which treated up to the main parameters of Chinese standard (GB 16889-2008) (MEP, 2008), such as chemical oxygen demand (COD) and NH4-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 the main parameters of Chinese discharge standard were investigated. The degradation of phthalic acid esters (PAEs) (typical endocrine disrupting chemicals, 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. The landfill has been run for more than 14 years and about 1,000–1,500 m3 of leachates are disposed of 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 and homogenized by mingling into glass containers. Prior to analysis, 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 the UV-Fenton process. The experiment comprised pH adjustment (pH: 2.3), and addition of solid iron sulfate heptahydrate (FeSO4·7H2O, 2 g/L) and hydrogen peroxide solution (H2O2, 4% v/v). At every time point, samples were moved from the photoreactors into the flask beakers for testing or storage 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 iron. All experimental procedures for Fenton treatment were the same except the 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 under oscillation (200 rpm) at a temperature of 20 °C. At every time interval, activated carbon was separated from the solution, before being further moved into flask beakers for testing or storage until analysis at 4 °C.

D. magna acute toxicity assays

D. magna acute toxicity assays were conducted, referring to US Environmental Protection Agency (US EPA 2002). Five concentrations (6.25, 12.5, 25, 50, and 100%) of untreated and treated MCs were chosen. The EC50 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% CO2.

Cytokinesis-block micronucleus (CBMN) assays were performed referring to a previous publication (Wang et al. 2016a). Sample preparation of untreated and treated MCs referred to the publication, mainly regarding 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, 0.5 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 was calculated according to the equation: CBPI = (M1 + 2M2 + 3Mn)/N, and M1, M2, Mn represented the number of cells with one, two, or multiple 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 referred to a previous publication (Gong et al. 2014), mainly regarding 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% CO₂.

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 an 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 were exposed to several concentrations of exposure medium for 48 hours. Internal positive controls, with PBS, and then were exposed to several concentrations of untreated and treated MCs, dimethylsulfoxide (DMSO) were conducted for all assays. To each well was added 20 μL (5 mg/L) of MTT after 48 hours, and this was continued to be cultured for 4–6 hours until infected media were discarded. A DMSO solution of 150 μL was added to each well, before vibrating for 10 min at a low speed in the dark to dissolve completely. The optical density (OD) of each well at the wavelength of 490 nm was detected using a 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:

\[
PE = \frac{\text{OD of experimental groups}}{\text{OD of negative controls}}
\]

**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 a single standard water sample (DMP of 200 μg/L, DBP of 200 μg/L, DEHP of 200 μg/L) and a 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 a mixed standard samples were treated with the same UV-Fenton process for MCs. The chemical pathways of PAEs changing in ESS were analyzed by gas chromatography–mass spectrometry (GC/MS) with some adjustments based on references (Kuch et al. 2010; Li et al. 2014) during the UV-Fenton process. At different setting times of 0, 5, 10, 15, 30, and 60 min, 80 ml samples were removed from the photoreactor and divided into two parts. Sodium chloride at 3 g was added to the 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 for the sampling times. The physical and chemical characterization of untreated MCs are listed in Table 1. Untreated MCs was a brown liquid, with...
a very low value of BOD₅/COD = 0.055, which leads to a low biodegradability. It had a nearly neutral pH and contained high concentration of Cl⁻ (2925.7 mg/L), CODₜ (724 mg/L) and conductivity (11.3 ms/cm).

The removal efficiency of CODₜ after UV-Fenton, Fenton and activated carbon adsorption treatment

Figure 1 shows the removal efficiency of CODₜ after UV-Fenton, Fenton and activated carbon adsorption processes. After 120 min Fenton and UV-Fenton process, the CODₜ of the concentrate decreased to 69.1% and 80.1% respectively. UV-Fenton had a better removal efficiency for CODₜ than Fenton treatment because UV radiation was able to 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 CODₜ 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 are presented in Table 2. The calculation of the EC₅₀ value was based on the sigmoidal concentration-response curves fitted by the least-squares methods (Ribé et al. 2012). Untreated MCs showed an acute toxic effect on D. magna with an EC₅₀ 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 to leachate toxicity (Chen et al. 2015). Concentrates treated with UV-Fenton at a time point of 30 min showed an obvious toxicity reduction (EC₅₀ = 23.5%, p<0.05). On the contrary, the acute toxic effect significantly increased (EC₅₀ = 7.28%, p<0.01) after 30 min Fenton process. This might be due to the Fenton oxidation of complex organic contaminants not resulting in a fast mineralization, with formation of carbon dioxide and inorganic species, but more poisonous oxidation intermediate products having 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 of 10, 20, 30, and 40 min showed respective EC₅₀ 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 shows the mean value of CBPI and MN resulting from HepG2 cells exposed to treated and untreated MCs.
Figure 2(a) clearly shows that untreated MCs could result in an obvious induction of the appearance of MN, even at the diluted concentration of 5% \((p < 0.05)\). Moreover, with the increase in 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). A high concentration of refractory organics (chromaticity, COD) might cause the genotoxicity (Gajski et al. 2012). Compared with negative controls, UV-Fenton treatment effluents at different concentrations showed no obvious difference in the number of MN. On the other hand, Fenton treatment effluents at different concentrations showed a slight increase in 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 number of micronuclei showed a significant difference compared with a negative control \((p < 0.05)\). With the concentration of activated carbon adsorption treatment effluent increasing, the numbers of micronuclei increased. Furthermore, samples treated by the 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 the UV-Fenton process had the highest removal efficiency for genotoxic matter. Meanwhile, the Fenton and activated carbon adsorption treatment effluents after 120 min still

<table>
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<th>Treatment time (min)</th>
<th>UV-Fenton</th>
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<th>AC</th>
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<tr>
<td>0</td>
<td>15.04</td>
<td>15.04</td>
<td>15.04</td>
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<tr>
<td>10</td>
<td>–</td>
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<td>20</td>
<td>23.5</td>
<td>7.28</td>
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<td>60</td>
<td>25.1</td>
<td>26.3</td>
<td>40.8</td>
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AC: activated carbon.

Table 2 | EC50 values of D. magna exposed to treated MCs

Figure 2 | Micronucleus assay results of HepG2 cells exposed to treated and untreated MCs for 24 h; MMC: Mitomycin C \((0.3 \mu \text{g/mL})\), 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.
showed some genotoxic effects because the formation rate of hydroxyl free radicals in the Fenton process was relatively slow and the activated carbon adsorption was nonselective and easily adsorption saturated.

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

Figure 3 shows 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 in untreated MCs concentration (Figure 3(a)). MCs treated by Fenton, UV-Fenton or activated carbon adsorption showed a reduction of %DNA in the tail to some degree compared with untreated MCs. But the %DNA in the 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 a slight increase compared with the negative control (Figure 3(c)). And 11.85 ± 2.53% of DNA in the 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 the three treatment methods, only UV-Fenton treatment effluent showed no obvious genotoxicity in these two assays, even though the parameters of the latter two treatment effluents, such as COD and NH₄-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 are shown 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, a further reduction of concentration resulted in the decrease of PE due to the reduction of estrogenic matters in MCs. After 60 min UV-Fenton or 90-min Fenton treatment, the PE of effluents

![Figure 3](https://iwaponline.com/wst/article-pdf/76/11/2949/210029/wst076112949.pdf)
showed no significant difference compared with the negative control. By contrast, the PE value of the final activated carbon adsorption treatment effluent was 110%, which 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 obvious estrogenicity in the above experiment, which indicated that there were EDCs in untreated MCs. So common EDCs, including DMP, DBP and DEHP (all are common PAEs), detected in landfill leachate, which had estrogenic effects and could cause health problems in humans and animals (Kuch et al. 2016), 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 (C₀/Cₜ) and UV-Fenton treatment duration are 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 r² 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 (C₀/Cₜ) 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 PAEs remaining in MCs, and further result in estrogenicity.

**Analysis of intermediates derived from the ESS UV-Fenton degradation process**

The total ion chromatogram of ESS prior to and after the UV-Fenton process for 10 min with GC/MS analysis is given in Figure 6. Since the 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: 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 generation from AOPs gave priority to attack the side chain of DBP and formed

![Degradation kinetic fitting curves of different phthalic acid esters during UV-Fenton process; C₀: concentration at time point 0 min, Cₜ: concentration at time point t min.](image)

**Figure 5** Degradation kinetic fitting curves of different phthalic acid esters during UV-Fenton process; C₀: concentration at time point 0 min, Cₜ: concentration at time point t min.

![Intermediates analysis results tested by total ion current gas chromatogram on GC/MS; (a) untreated, (b) treated by UV-Fenton process for 10 min. Peaks 1, 2 and 3 represent DMP, DBP, DEHP respectively. Peaks 4–13 represent other compounds formed in the UV-Fenton process. The arrows represent peak areas increasing significantly compared with that of UV-Fenton treated for 5 min.](image)

**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. Peaks 1, 2 and 3 represent DMP, DBP, DEHP respectively. Peaks 4–13 represent other compounds formed in the UV-Fenton process. The arrows represent peak areas increasing significantly compared with that of UV-Fenton treated for 5 min.
Mono-n-butyl phthalate and DEP (Wu et al. 2015). 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 peaks 9, 12 and 13 were more complex than peak 2 of DBP and simpler than 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 there being no matched structures in the standard mass spectrum library and more complex chemical structures, other intermediates could not 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 the UV-Fenton oxidation process might be one of the reasons for 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 CODcr of the effluents was 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 the 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 reach a satisfactory level and the regeneration of activated carbon was still a problem, considering the cost. Dynamics and intermediates analysis of PAEs degraded by the 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. 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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