Chemosphere 180 (2017) 192–200

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Estrogenicity assessment of membrane concentrates from landfill leachate treated by the UV-Fenton process using a human breast carcinoma cell line

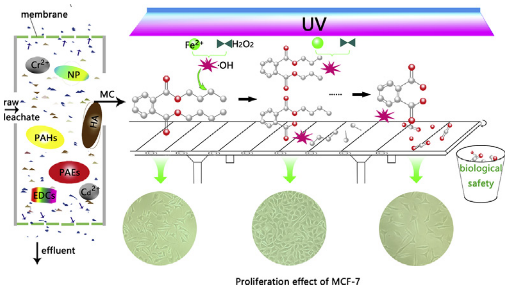
Changcheng Hou [a], Gang Lu [a, *], Ling Zhao [a, **], Pinghe Yin [b], Lingfei Zhu [a]

[a] School of Environment, Guangzhou Key Laboratory of Environmental Exposure and Health, Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou 510632, China
[b] Research Center of Analysis and Test, Jinan University, Guangzhou 510632, China

HIGHLIGHTS

- Membrane concentrates induce estrogenicity in MCF-7 cells and threaten human and environmental health.
- PAEs were refractory and a main contributor to membrane concentrate estrogenicity.
- The degradation intermediates were more toxic than the original toxic substances in membrane concentrates.
- Estrogenicity was highly related to the generation of intermediates and the concentration of PAEs.
- Estrogenicity assessment was an effective method for evaluating the AOPs of membrane concentrates.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 January 2017
Received in revised form
6 April 2017
Accepted 8 April 2017
Available online 8 April 2017

Handling Editor: Frederic Leusch

Keywords:
Estrogenicity assessment
Intermediate analysis

ABSTRACT

Membrane concentrates (MCs) are generated when membranes are used to concentrate landfill leachate. It contains high concentrations of inorganic and organic environmental pollutants, which are highly toxic and carcinogenic. In this paper, the proliferation effect (PE) from MC before and after treatment with the UV-Fenton process was assessed using the human breast carcinoma cell line MCF-7. The highest value of 116% was found at 5% (v/v) concentration after a 10 min reaction. Phthalic acid esters (PAEs) play an important role in the MC estrogenicity. Estrogen simulation solutions (ESS) of PAEs were prepared to simulate the changes in estrogenic active substances during the UV-Fenton process. The ESS degradation conformed to the first-order kinetics model. The estrogenicity decreased after an initial increase until it acted in a non-estrogenic manner. Convincingly, the intermediates were determined by GC/MS, and the estrogenicity was assessed during the degradation process. The estrogenicity was highly related to the

---

* Corresponding author.
** Corresponding author.
E-mail addresses: tlugang@jnu.edu.cn (G. Lu), zhaoling@jnu.edu.cn (L. Zhao).

http://dx.doi.org/10.1016/j.chemosphere.2017.04.033

Advanced oxidation process
Membrane concentrates of landfill leachate
Endocrine disrupting chemicals

generation of intermediates and the PAE concentration. The results provide guidance for UV-Fenton application in MC estrogenicity reduction.

1. Introduction

Landfill leachate, a highly concentrated organic wastewater, is generated by decomposition of organic wastes and infiltration of rainfall (Paskuliakova et al., 2016; Long et al., 2017). Membrane-based treatment processes, including ultrafiltration, nanofiltration, and reverse osmosis (Kosutic et al., 2015; Li et al., 2016a) generate a large volume of membrane concentrate (MC) (He et al., 2015). The MCs often contain high concentrations of endocrine disrupting compounds (EDCs) (Li et al., 2016a; Husain and Qayyum, 2013; Lewis et al., 2012), which is becoming one of the most problematic issues during landfill operation. Therefore, the MC must be treated thoroughly before it is discharged into receiving environments. Although the concentrations of chemical oxygen demand (COD) and certain compounds in the effluent meet discharge standards, some trace level EDCs in the effluent have the potential to cause estrogenicity (Lewis et al., 2012).

MC has a high level of EDCs, which cause a high potential estrogenicity risk to the ecosystem. EDCs such as phthalic acid esters (PAEs), polycyclic aromatic hydrocarbon (PAHs), bisphenol A (BPA), nonylphenol (NP), and heavy metals are ubiquitously contained in daily necessities, resulting in high concentrations of EDCs in landfill sites (Kalmykova et al., 2013; Inoue et al., 2016; Jin and Zhu, 2016). Thus, the EDCs in the leachate could challenge the capacity of existing treatment methods. Although the target estrogens were partially removed by the “membrane bioreactor + aeration zeolite biofilter” treatment process, the treated leachate was still estrogenic to the ecosystem (Gong et al., 2014; Haig et al., 2016). The estrogenicity within the range from below the detection limit to 29 ng L^{-1} estradiol was still found in the treated leachate (Svenson et al., 2011). Low concentrations of EDCs have carcinogenic potential. In addition, trace levels of contaminants present in leachate were toxic enough to cause synergistic or additive cytotoxicity and affect human health (Ghosh et al., 2015).

At present, the main technologies for MC treatment include evaporation (Benyoucef et al., 2016), coagulation (Amor et al., 2015), recirculation to landfills (Xing et al., 2013), and advanced oxidation processes (AOPs) (He et al., 2015; Talalaj, 2015). Amongst them, AOPs are gaining importance as an alternative to the biological and traditional physicochemical treatments for leachate (Oulego et al., 2016). The UV-Fenton process (a combination of hydrogen peroxide, UV radiation, and ferrous iron) is an example of an AOP, which can degrade persistent organic pollutants by the action of hydroxyl radicals (Amor et al., 2015; Umar et al., 2010). It is the most effective approach among the H_2O_2-based AOPs to enhance the biodegradability and eliminate the color of the MC (Wang et al., 2016a). Thus, it is widely used in wastewater treatment plants (Iqbal et al., 2017; Silva et al., 2017). Additionally, it provides toxicological safety for treating effluent (Wang et al., 2016b) because it oxidizes the organic matter into terminal products such as H_2O, CO_2, and other mineralization materials. However, the cost of treating effluents to non-toxicity is significantly high, resulting in incompletely mineralized effluents and discharge with estrogenicity in real wastewater treatment (Oulego et al., 2016).

COD and EDC concentration limits are used as standards for wastewater discharge. After AOP treatment, the MC meets these standards and can be discharged properly (Ren et al., 2016). However, when the EDCs were transformed into intermediates or mixed with other compounds, the measured concentration did not represent the estrogenicity correctly. Thus, there was estrogenicity remaining in the effluents (Wang et al., 2016a). Although EDCs were below the detection limit after treatment, the estrogenicity contribution to the ecosystem should be a focus area. A human breast carcinoma cell line, MCF-7, can be used to assess estrogenicity. It expresses estrogen and progesterone receptor genes, which may comprehensively reflect the estrogenicity of all EDCs in the environment (Li et al., 2016b; Vantangoli et al., 2016). These molecular characteristics provide references for the epigenetic study of breast cancer (Johnson et al., 2014). The MCF-7 flow-cytometric proliferation effect (PE) assay was used to establish potential estrogen-disrupting effects (Krivoshiev et al., 2016; Papa et al., 2016).

The aim of this study was to assess the changes in estrogenicity during the UV-Fenton processing of MC using the human breast carcinoma cell line, MCF-7. Meanwhile, the main EDCs in MC, degradation intermediates, and kinetics of the EDCs during the AOPs were investigated by GC/MS. The estrogen simulation solutions (ESS) were used to stimulate the EDCs in MCs to study the structures and estrogenicity performance of the intermediates.

2. Materials and methods

2.1. Sample description and preparation

The MCs were collected from a leachate treatment plant in Xiaping, Shenzhen, China in operation since 2002. The landfill covers an area of 1 490 000 m^2 and disposes approximately 1000–1500 m^3 leachate per day. The detailed flow diagram for the raw landfill leachate treatment process is given in Fig. 1. The leachate was mixed uniformly in regulation tanks, and H_2SO_4 or NaOH was added to adjust the pH in the reaction sedimentation tank. After treatment with an ammonia stripping tower and the subsequent expanded granular sludge bed (EGSB) process to remove COD, the treated leachate entered a combined processing area including the A/O/O (anaerobic/oxic/oxic) reactor, which efficiently denitrifies the leachate, and the Membrane Bio-Reactor (MBR) (Ogunlaja and Parker, 2015; Silva et al., 2013). In conclusion, passage through the denitrification, MBR, and nanofiltration membranes (NF90-400, USA) satisfied effluent discharge standards, whereas the final nanofiltration MCs were collected and stored pending subsequent processing (Silva et al., 2013).

Each 5 L sample was collected weekly during April 2015 and mixed in glass containers. All samples were stored at 4 °C before analyzing. Physicochemical parameters were measured immediately after all samples were collected.

2.2. Advanced oxidation experiments

The UV-Fenton process was conducted in a 1.2-L container with ultraviolet light (predominant UV intensity at 253.7 nm, 75 mW cm^{-2} at the surface of the lamp) and aeration devices. The MC pH was adjusted up to 3.0 with the slow addition of H_2SO_4

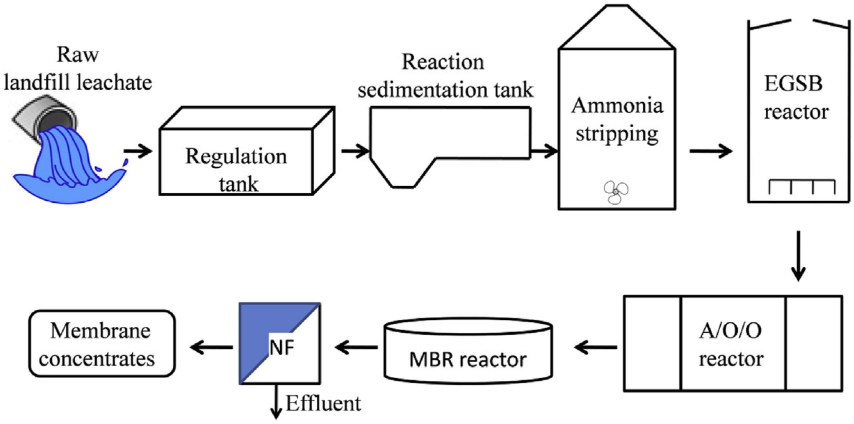


Fig. 1. Schematic diagram of leachate treatment process.

solution (50% v/v). Two grams of solid iron sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) were added to make a concentration of 2 g L^{-1}. The concentration of hydrogen peroxide solution (H_2O_2, 30% w/w) was adjusted to 100 mM. In order to terminate the reaction and precipitate the remaining iron, 80 mL of each sample was taken out after a designated reaction time (0, 5, 10, 30, or 60 min) and neutralized with NaOH solution (50%, w/w) to adjust the pH up to 9.0. Then, each sample was filtered through a 0.4-μm membrane and stored at 4 °C until analysis. The negative control experiment (no ultraviolet light or H_2O_2) was performed under the same conditions (Cedat et al., 2016; Li et al., 2016c; Boonnorat et al., 2016).

According to the GC/MS and ICP/MS computer library for quantitative analysis, some MC indicators from the Xiaping treatment plant were determined with credibility up to 80%. The specific pollutant detection methods were: concentration of pollutants in the MC, the standards of benzo(*a*)pyrene (BaP) (Ghosh et al., 2015), lead ions (Pb^{2+}), cadmium ions (Cd^{2+}), hexavalent chromium (Cr^{6+}) (Panagiotakis et al., 2015), bisphenol A (BPA) (Yiu et al., 2014), PAEs (DMP, DBP, and DEHP) (Boonnorat et al., 2014), and NP (Loffredo and Castellana, 2015), which were selected to conduct the PE evaluation using MCF-7 cells under laboratory conditions. In particular, humic acid was separated from the MC by a XAD-8 resin of Amberlite (Sigma-3025-U, USA) (Nwosu and Cook, 2015).

ESS Preparation. 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 (0.1000 g), DBP (0.1000 g), or DEHP (0.1000 g) were put into 100-mL volumetric flasks to prepare 1000 mg L^{-1} single standard stock solutions with methanol and stored in the dark at 4 °C before use. The stock solutions were used to prepare a standard single sample (DMP 200 μg L^{-1}, DBP 200 μg L^{-1}, and DEHP 200 μg L^{-1}) and mixed with a standard water sample. The standard samples were used as the ESS and treated with the same UV-Fenton process as the MC.

2.3. Analysis of intermediates

The chemical pathways for PAE degradation in MC and ESS were analyzed by GC/MS with some adjustments based on references (Haig et al., 2016; Li et al., 2014; Kuch et al., 2010) during the UV-Fenton process. At different settling 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 (3 g) was added to the 50-mL sample to prevent its emulsification, and the sample was extracted three times with 20 mL dichloromethane by liquid–liquid extraction in a 150-mL separator funnel. The organic phase was collected, dried by anhydrous sodium sulfate, and evaporated to dryness using a rotary evaporator. Finally, the product was diluted with hexane to 1 mL. The concentration of total organic carbon (TOC) in the remaining 30-mL sample was determined with the vario TOC cube (ELEMENTAR, Germany), which could roughly reflect the mineralization level of the water sample.

2.4. Estrogenicity test

MCF-7 cells were purchased from the First Affiliated Hospital of Jinan University and were grown in Dulbecco's modified Eagle's medium (DMEM) without phenol red, but supplemented with 10% non-hormone fetal bovine serum and 1% penicillin/streptomycin (1000 U mL^{-1} penicillin and 1000 U mL^{-1} streptomycin). Cells were maintained in an incubator at 37 °C in a humidified atmosphere with 95% air and 5% CO_2 (Johnson et al., 2014; Li et al., 2016c; Buteau-Lozano et al., 2008; Olmez-Hanci et al., 2015).

After the extraction process in section 2.3, dimethylsulfoxide (DMSO) was added as a solvent replacement to generate the exposure solution for the UV-Fenton processing of the ESS. The PE was evaluated with methyltetrazolium (MTT) (Wang et al., 2016b; Li et al., 2016b). After culturing in a 96-well plate (10^4 cells/well) for 24 h, non-hormone MCF-7 cells were washed twice with PBS and exposed to several concentrations of exposure medium (ranging from 10^{-5}–10^2 mg L^{-1}, diluted with DMEM) for 48 h. Negative controls (culture medium, MTT, and DMSO) and controls (cells, culture medium, MTT, and DMSO) were conducted at the same time. Three parallel independent experiments were set up for each test. MTT (20 μL, 5 mg L^{-1}) was added to each well after 48 h and cells continued to be cultured for 4–6 h until infected media were discarded. DMSO solution (150 μL) was added to each well and vibrated for 10 min at a low speed in the dark until completely dissolved. The optical density (OD) of each well at the 490-nm wavelength was detected using an automatic microplate reader (Multiskan MK3, Thermo Fisher, USA). The PE was calculated with the following formula (Johnson et al., 2014; Kuch et al., 2010; Li et al., 2016d):

PE= (experimental group - negative controls)/(controls - negative controls) × 100%

2.5. Statistical analysis

All experimental data were expressed as mean ± standard deviation. The statistical differences in the PE test parameters between control and tested samples were examined by analysis of variance (ANOVA). The statistical significance was set at $P < 0.05$.

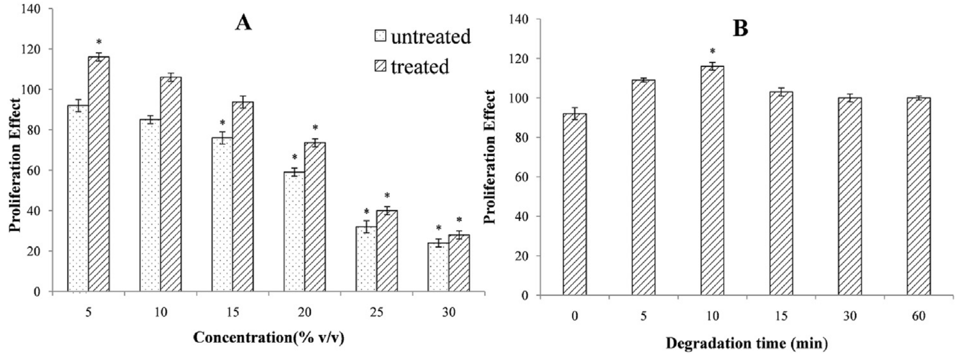


Fig. 2. MCF-7 cell proliferation effect (PE) after 48-h exposure to different membrane concentrate (MC) untreated/treated concentrations and degradation times, 100% = control. (A) Cell PEs from different concentrations of untreated and UV-Fenton treated MCs for 10 min; (B) Cell PEs of different treatment times with UV-Fenton (5% v/v). Values represent mean ± SD, n = 3. *Statistically significant compared to negative control ($P < 0.05$).

3. Results

3.1. PE analysis of MC

MCF-7 cells were exposed to the untreated and treated MC. The PE in the MCF-7 cells following exposure is presented in Fig. 2. The PE was expressed as a percentage of the corresponding control group. Exposure to untreated MC caused a significant reduction in the PE. The highest tested concentration, 30% (v/v) MC, showed the lowest PE value (Fig. 2A). Compared to the control group, statistical significance was obtained ($P < 0.05$) for the concentrations ranging from 15 to 30% (v/v). MCF-7 cells exposed to treated samples after 10 min of UV-Fenton processing showed higher cell activity. In the low concentration of 5% (v/v), the PE increased to 116% compared to the control group. Fig. 2B depicts the increase in the PE over degradation time. The PE increased gradually until reaching the maximum of 119% at 10 min, and then began to decrease. At 60 min, it showed no significant difference from the control group.

3.2. Characteristics analysis of MC

Table 1 shows the basic physicochemical parameters and the PE of the MC. The concentrations of Pb^{2+} (20 μg L^{-1}) and Cd^{2+} (69 μg L^{-1}) were too low to cause cell proliferation compared with the control group in 48-h cultures. BPA caused higher PEs than NP and BaP in the MC, but lower than the Cr^{6+}. In particular, PAEs caused a more significant cell PE, up to 119%, compared to the control group, and just below the E_2 positive control of 142%. In addition, the results presented in Table 1 suggest that PAEs contribute to the MC estrogenicity, and the UV-Fenton process could efficiently remove pollutants in the MC and reduce the estrogenicity.

3.3. Dynamics analysis

Fig. 3A simulates the correlation of C_t/C_0 with time during the UV-Fenton process. The ultimate degradation efficiency of DMP, DBP, and DEHP was 98.7%, 93.6%, and 89.4%, respectively. Thus, the DMP was easier to degrade than the DBP, whereas the DEHP was more difficult to degrade than the DBP. Fig. 4B verified that the linear correlation coefficients (r^2) of the DMP, DBP, and DEHP were 0.953, 0.976, and 0.962, respectively. Thus, there was a significantly linear relationship between (C_0/C_t) of the three PAEs and the degradation time during the UV-Fenton process, consistent with the first-order kinetics model. From the slopes of the degradation kinetic fitting curves, it could be inferred that the half-life of DMP, DBP, and DEHP was 6.37 min, 8.57 min, and 9.85 min, respectively.

Table 1
Physicochemical characteristics of untreated and treated membrane concentrate (MC) from the landfill site of Xiaping (Shenzhen, Guangdong, China) and MCF-7 proliferation effect (PE) for the main MC pollutants.

Parameter	Untreated		Treated	
	Concentrate	PE(%)	Concentrate	PE(%)
Color (PtCo)	5300	–	26	–
pH	7.9	–	9	–
Turbidity (NTU)	3900	–	25	–
EC[a] (Ms/cm)	22.7	–	17.9	–
COD_{Cr}[b] (mg/L)	2189	–	113	–
HA[c] (mg/L)	965	n.d[d]	n.d	n.d
Pb^{2+}(μg/L)	20	n.d	n.d	n.d
Cd^{2+}(μg/L)	69	n.d	n.d	n.d
DMP(μg/L)	127	115	n.d	n.d
DBP(μg/L)	215	119	n.d	n.d
DEHP(μg/L)	197	117	1.3	n.d
BPA(μg/L)	8.6 ± 2.3	107	n.d	n.d
NP(μg/L)	6.8 ± 0.9	104	n.d	n.d
BaP(μg/L)	18.4 ± 3.2	103	n.d	n.d
Cr^{6+}(μg/L)	114	109	n.d	n.d

[a] Electrical conductivity.
[b] Chemical oxygen demand.
[c] Humic acid.
[d] Below detection limit.

3.4. Concentration analysis

The PE values of MCF-7 cells exposed to different concentrations of PAEs in untreated and treated ESS at 10 min are shown in Fig. 4. In untreated ESS, a high concentration of EDCs resulted in inhibition of the MCF-7 cell PE. The DMP showed a relatively lower PE inhibition rate with 89% than that of the DEHP with 45% and the DBP with 9%. The inhibition effect appeared more obvious at higher concentrations of PAE mixtures ranging from 10^0 to 10^2 mg L^{-1}. With the decrease in concentration, the PE increased and reached peak values of 109% for DMP, 124% for DBP, and 122% for DEHP. The peak values appeared at similar concentrations from 10^{-2} to 10^{-1} mg L^{-1} compared to the control groups. Afterwards, the PE began to decline until MCF-7 cells showed normal cellular activity. Thus, there was a relationship between the concentration and the PE of PAEs. PAE mixtures showed a similar trend, as well. The peak PE value was 131% at 10^{-2} mg L^{-1} after UV-Fenton treatment for 10 min, which was much higher than those from single PAEs.

3.5. Intermediate analysis

Fig. 5 shows the total ESS ion chromatogram before and after UV-Fenton processing for 10 min with GC/MS analysis. Because the

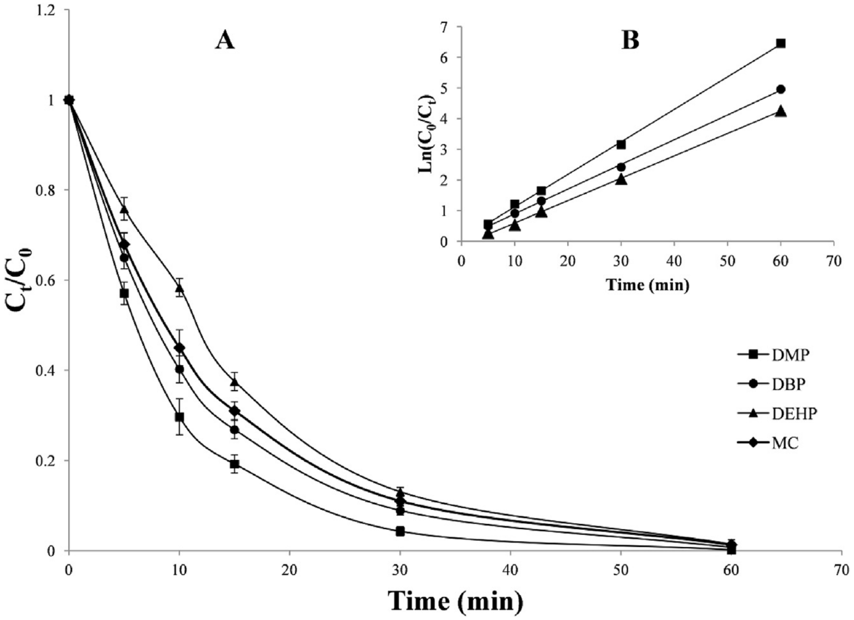


Fig. 3. Degradation kinetic curves of different PAEs using the UV-Fenton process. (A) C_t: the concentration at the moment t min, C_0: the concentration at the moment 0 min; (B) Degradation kinetic fitting curves. The data were collected from three independent experiments and are presented as the means ± SD.

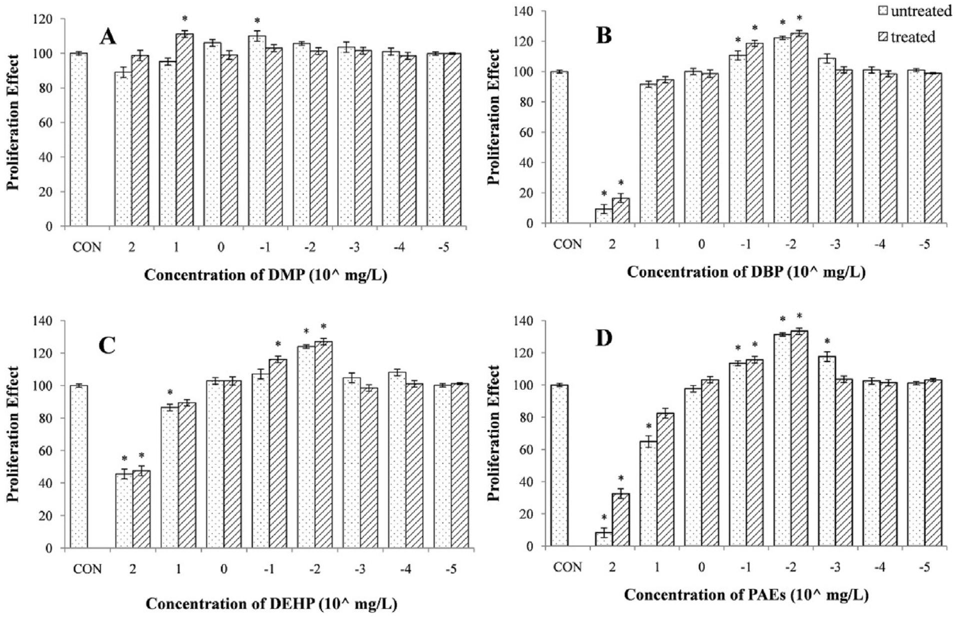


Fig. 4. Proliferation effect (PE) in MCF-7 cells after 48-h exposure to different concentrations of untreated and treated ESS for 10 min (A) DMP; (B) DBP; (C) DEHP; (D) PAEs. CON = control. The control cells were incubated with 0.1% dimethylsulfoxide (DMSO). The PE was determined by the MTT assay and calculated as the percentage of control viability. The data are presented as the means ± SD from three independent experiments. *$P < 0.05$ vs. the control.

peak areas of 1, 2, and 3 representing the DMP, DBP, and DEHP, respectively, had decreased, all three PAEs could be degraded by the UV-Fenton process. The peaks 4–13 represented the intermediates captured in the degradation process. Standard mass spectrum searches and characteristic ion peak analyses revealed that peak 5 (retention time: 7.85 min, *m*/*z*: 222 149) and peak 6 (retention time: 8.71, *m*/*z*: 149 205) were butyl phthalate and diethyl phthalate, respectively. The DMP was detected in the single DBP degradation process. The structures of the substances represented by the peaks 9, 12, and 13 were more complex than the DBP peak 2 and simpler than the DEHP peak 3 because their retention times were longer than that of DBP and shorter than that of DEHP. Therefore, it was inferred that the three substances were 2-ethyl hexyl-*n*-butyl phthalate, diisophenyl *ortho*-phthalate, and benzyl-*n*-butyl *ortho*-phthalate, respectively. The other intermediates could not be confirmed because no matched structures were found in the standard mass spectrum library and the chemical structures were complex.

3.6. *Degradation phase analysis*

Fig. 6 indicates the ESS estrogenicity variation at the

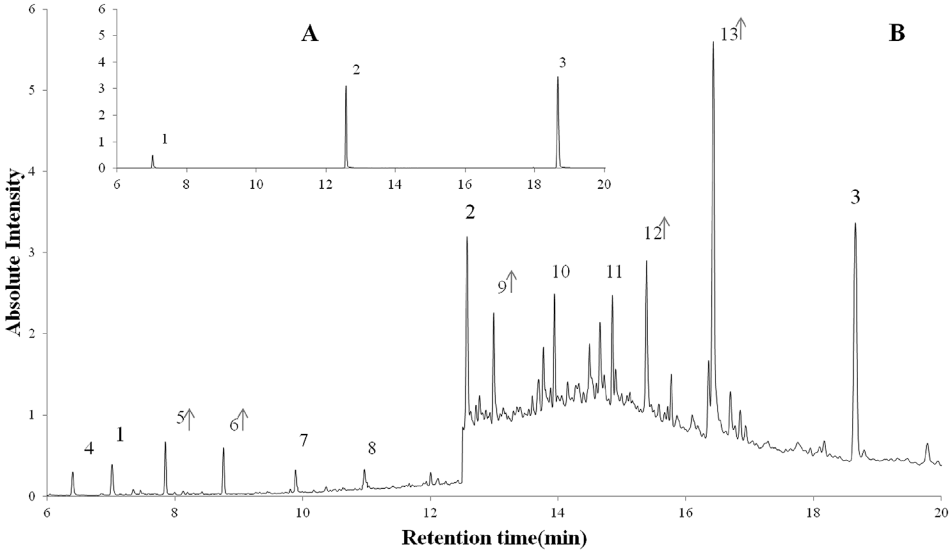


Fig. 5. Characterization of the intermediates by total ion current gas chromatogram on GC/MS. (A) Untreated; (B) treated with the UV-Fenton process for 10 min. Peaks 1, 2, and 3 represent DMP, DBP, and DEHP, respectively. Peak 4–13 represent other compounds produced in the UV-Fenton process. The arrows represent peak areas increased significantly compared to 5 min UV-Fenton treatment.

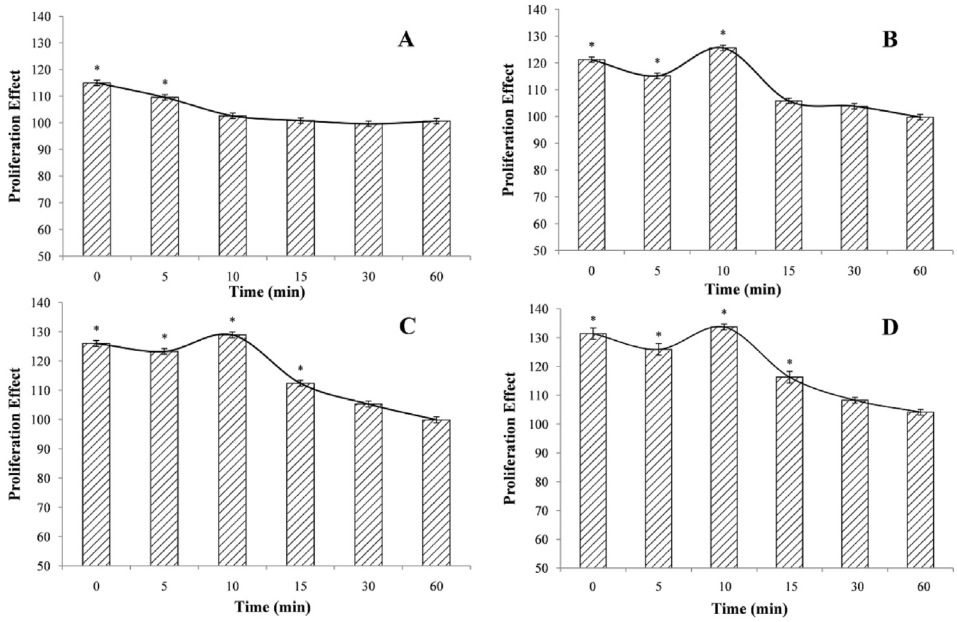


Fig. 6. The estrogenicity variation after the UV-Fenton degradation processes in the estrogen simulation solutions (ESS). (A) DMP; (B) DBP; (C) DEHP; (D) PAEs; The control cells were incubated with 0.1% dimethylsulfoxide (DMSO). The proliferation effect was determined by the MTT assay and calculated as the percentage of control viability. The data are presented as the means ± SD from three independent experiments. $^{*}P < 0.05$ vs. the control.

concentration 10^{-2} mg L^{-1} during the UV-Fenton process. The DMP was easier to degrade because of its relatively simple structures, so its PE declined gradually from 0 to 60 min and showed no estrogenicity after 60-min degradation. The PE values of other PAEs were higher than that of the DMP and showed various trends. The PE of DBP was 126%, whereas the PE of DEHP was 121% at 0 min. Unusually, after a 10-min degradation, the PE of DBP was increased to 127% and the DEHP to 125%, higher than that at 0-min degradation. Subsequently, the PE of DMP declined rapidly, while the PE of DBP and DEHP declined gradually. The PAEs mixture showed a similar trend as the DBP and DEHP, but the maximum PE value was 133% at 10 min. After treatment by the UV-Fenton process for 60 min, all the PE values from the PAEs showed no statistically significant differences compared to the control group.

4. Discussion

MCs are a new wastewater categorized as hazardous waste. It is not only a huge threat to the ecosystem, but also causes serious harm to human health (Haig et al., 2016). A new and effective technology is needed to treat MC. It was demonstrated that the UV-Fenton process could effectively reduce or remove cytotoxicity in MC compared to the Fenton and other processes (Wang et al., 2016b). MC contains a high concentration of EDCs and salts, and remarkable estrogenicity was detected in previous studies (Oturan et al., 2015; Coors et al., 2003). In this study, the PE value was dependent on the degradation time. The MC of lower concentrations (5% v/v) degraded by the UV-Fenton process for 10 min showed the highest estrogenicity effect on MCF-7 cells (Fig. 2). The highest concentration of 30% MC inhibited cell growth and led to the lowest PE. With the concentration decrease in MC, the inhibition effect weakened, whereas the PE value increased, explaining why the treated MC showed a higher PE value than the untreated MC.

Landfill leachate estrogenicity is mainly from highly aromatic organic matters and dissolved organic matters, which are hydrophobic acids (Zhang et al., 2009; Molins-Delgado et al., 2016). In this study, the estrogenicity of the main pollutants in MC was assessed by MCF-7 cells and presented in Table 1. PAEs showed the highest PE value. In fact, among all the detected EDCs in the MC, the concentration of PAEs was the highest. The reason might be that PAEs were widely used as a plasticizer in recent decades (Kalmykova et al., 2014). Moreover, PAEs were refractory organic matter in MC and scarcely degraded by biodegradation. The high concentrations of PAEs and the PE values of MCs observed in this study were similar to those of MCs found in other studies (Boonnorat et al., 2014).

The selected chemicals, including DMP, DBP, and DEHP, are common EDCs in landfill leachate with estrogen-like effects causing health problems in humans and animals (Kuch et al., 2010). However, EDCs are hard to remove thoroughly using traditional leachate treatments (Cedat et al., 2016). In this study, UV-Fenton was used to degrade the ESS. The hydroxyl free radicals generated during the AOPs could be used to degrade PAEs (Li et al., 2016a; Xu et al., 2010; Garcia-Segura et al., 2013). The degradation of PAEs in the ESS occurred according to the first order kinetic equation with an ultimate degradation efficiency up to 90% (Fig. 3). Similarly, in the MC, PAEs were also found to comply approximately with first order kinetics. The UV-Fenton process is a feasible tool for removing PAEs.

Although satisfactory results from the UV-Fenton processing of PAEs were obtained, the cell proliferation toxicity of this treatment process needed to be further studied. Generally, the estrogenicity of PAEs decreases as the concentration decreases. An interesting phenomenon showed that with the 10 min UV-Fenton process, the concentration of PAEs decreased significantly (Fig. 3), but the PE values showed an abnormal increase. By studying the ESS of PAEs ranging from 10^{-5}–10^{2} mg L^{-1}, it was found that the PE varied at different concentrations. Other studies report that the PE is highly dependent on the concentrations of PAEs (Ma et al., 2016). In Fig. 4, all the PAEs without UV-Fenton treatment, ranging from 10^{-2} to 10^{-1} mg L^{-1}, showed the highest estrogenicity and the PE values differed for the PAEs with different side chains, such as DMP, DBP, and DEHP. At the level of 10^{-2} mg L^{-1}, the PE order was mixed PAEs > DBP > DEHP > DMP, in accordance with the results in other studies (Huo et al., 2016). In addition, the inhibition effect appeared when the concentrations of all the PAEs were higher than 10 mg L^{-1} and no significant PE was observed below 10^{-4} mg L^{-1}. Therefore, the untreated MC at the highest concentration of 30% showed a lower PE than that of 5% because of the inhibition effect. As shown in Table 1 and Fig. 2, the initial PE of MC increased to the highest value and then decreased until it appeared non-estrogenic. The initial PAEs concentration was up to 1 mg L^{-1}, so the inhibition effect was dominantly present. As the PAE were degraded, the inhibition effect was weakened and was gradually substituted by the PE. Therefore, the PE gradually increased to its maximum and then declined to non-detectable levels with the degradation of estrogenic substances. In fact, cell PEs in MC during degradation were mainly caused by the original EDCs, but the product generated in the MC degradation process also might cause PEs. Therefore, changes in PE values only dependent on the decrease in the original EDCs concentration cannot explain this phenomenon.

The results from GC/MS analysis indicated that there were many new intermediate compounds when the ESS were degraded (Fig. 5). In this study, the phthalates peak (peak 4) was found in the degradation process, which conforms to previous studies (Chen et al., 2015). Based on standard spectrum library identification and mass spectrogram, peak 5 and peak 6 were determined to be mono-*n*-butyl phthalate and di-ethyl phthalate (DEP), respectively. Previous studies show that hydroxyl free radicals generated from AOPs first attack the side chain of DBP and form mono-*n*-butyl phthalate and DEP (Suzuki et al., 2001; Wu et al., 2015). The degradation of PAEs in the ESS started from the side chains and split into a variety of phthalate monoesters, further oxidizing into small-molecule substances such as carbon dioxide and water. Some intermediates were observed at peaks 9–13. The intermediates diisophenylortho-phthalate at peak 12 and benzyl-*n*-butyl *ortho*-phthalate at peak 13 showed obvious and stable increases in a time-dependent manner.

Thus, the PE of different degradation stages was determined. Because of its simple structure, DMP could be degraded easily and the PE value was reduced steadily, which was similar to previous studies (Zhang et al., 2009). However, the structures of DBP and DEHP are more complex. Therefore, during the DBP and DEHP degradations, intermediates were generated and accumulated during the initial 10-min degradation. As a result, the PE values of DBP and DEHP at the 10-min degradation time point were higher than that at the 0-min degradation time point (Fig. 6). The intermediates generated by DBP and DEHP degradation resulted in the PE increase. After degradation for 60 min, the PAEs were oxidized to simple small-molecule substances and ultimately showed non-estrogenicity. In addition, the PE variation trend from different degradation stages of the ESS conformed to the MC results (Figs. 2 and 6). Thus, the degradation process intermediates could lead to the initial estrogenicity increase during the AOPs process and be further oxidized by the numerous hydroxyl radicals in the subsequent degradation process. Considering this defect, the abnormal estrogenicity increase during the AOPs process should not be ignored and must be properly controlled in practical applications.

5. Conclusions

Our study presented the results of MC estrogenicity changes during the UV-Fenton process using a human breast carcinoma cell line (MCF-7). After UV-Fenton processing, the MC estrogenicity increased to the highest PE value, up to 119% of the MC value, at 10 min and gradually decreased to non-estrogenic levels. The heavy metals Pb^{2+} and Cd^{2+} were detected, but the concentrations were too low to cause estrogenicity, whereas the Cr^{6+} could cause a PE value of 109%. BPA, NP, and BaP were detected to be lower than 20 μg L^{-1}, much lower than the PAEs. Thus, the PAEs played an important role in the estrogenicity contribution of MC. The PAEs degradation conformed to the first-order kinetics model, and the degradation intermediates of butyl phthalate, diethyl phthalate, dimethyl *ortho*-phthalate, diisophenylortho-phthalate, benzyl-*n*-butyl *ortho*-phthalate, etc. were determined by GC/MS. The concentration changes of PAEs and the generation of intermediates had a great effect on the estrogenicity of the MC during the UV-Fenton process. The estrogenicity increase caused by the degradation intermediates during the AOPs must be considered. Furthermore, the estrogenicity assessment could be an auxiliary index for wastewater discharge in the future.

Competing financial interest

The authors declare no competing financial interest.

Acknowledgments

This study was financially supported by National Natural Science Foundation of China (Grant No. 51508228, 41676110), Industry-university-research combination project of Guangdong Province (2013B090600009), Pearl River S&T Nova Program of Guangzhou (201710010091), and science and technology projects of Guangdong province (2014A020217007).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2017.04.033.

References

Amor, C., De Torres-Socias, E., Peres, J.A., Maldonado, M.I., Oller, I., Malato, S., Lucas, M.S., 2015. Mature landfill leachate treatment by coagulation/flocculation combined with Fenton and solar photo-Fenton processes. J. Hazard. Mater. 286, 261–268.

Benyoucef, F., Makan, A., El Ghmari, A., Ouatmane, A., 2016. Optimized evaporation technique for leachate treatment: small scale implementation. J. Environ. Manage 170, 131–135.

Boonnorat, J., Chiemchaisri, C., Chiemchaisri, W., Yamamoto, K., 2014. Removals of phenolic compounds and phthalic acid esters in landfill leachate by microbial sludge of two-stage membrane bioreactor. J. Hazard. Mater. 277, 93–101.

Boonnorat, J., Chiemchaisri, C., Chiemchaisri, W., Yamamoto, K., 2016. Kinetics of phenolic and phthalic acid esters biodegradation in membrane bioreactor (MBR) treating municipal landfill leachate. Chemosphere 150, 639–649.

Buteau-Lozano, H., Velasco, G., Cristofari, M., Balaguer, P., Perrot-Applanat, M., 2008. Xenoestrogens modulate vascular endothelial growth factor secretion in breast cancer cells through an estrogen receptor-dependent mechanism. J. Endocrinol. 196, 399–412.

Cedat, B., de Brauer, C., Metivier, H., Dumont, N., Tutundjan, R., 2016. Are UV photolysis and UV/H2O2 process efficient to treat estrogens in waters? Chemical and biological assessment at pilot scale. Water Res. 100, 357–366.

Chen, B., Song, J.X., Yang, L.H., Bai, Q., Li, R.J., Zhang, Y.M., Rittmann, B., 2015. Coupling UV-H2O2 to accelerate dimethyl phthalate (DMP) biodegradation and oxidation. Biodegradation 26, 431–441.

Coors, A., Jones, P.D., Giesy, J.P., Ratte, H.T., 2003. Removal of estrogenic activity from municipal waste landfill leachate assessed with a bioassay based on reporter gene expression. Environ. Sci. Technol. 37, 3430–3434.

Garcia-Segura, S., Salazar, R., Brillas, E., 2013. Mineralization of phthalic acid by solar photoelectro-Fenton with a stirred boron-doped diamond/air-diffusion tank reactor: influence of Fe3+ and Cu2+ catalysts and identification of oxidation products. Electrochim. Acta 113, 609–619.

Ghosh, P., Gupta, A., Thakur, I.S., 2015. Combined chemical and toxicological evaluation of leachate from municipal solid waste landfill sites of Delhi, India. Environ. Sci. Pollut. Res. 22, 9148–9158.

Gong, Y.F., Tian, H., Wang, L.J., Yu, S.P., Ru, S.G., 2014. An integrated approach combining chemical analysis and an in vivo bioassay to assess the estrogenic potency of a municipal solid waste landfill leachate in qingdao. Plos One 9.

Haig, S.J., Gauchotte-Lindsay, C., Collins, G., Quince, C., 2016. Bioaugmentation mitigates the impact of estrogen on coliform-grazing Protozoa in slow sand filters. Environ. Sci. Technol. 50, 3101–3110.

He, R., Wei, X.M., Tian, B.H., Su, Y., Lu, Y.L., 2015. Characterization of a joint recirculation of concentrated leachate and leachate to landfills with a microaerobic bioreactor for leachate treatment. Waste Manage 46, 380–388.

Huo, C.Y., Liu, L.Y., Zhang, Z.F., Ma, W.L., Song, W.W., Li, H.L., Li, W.L., Kannan, K., Wu, Y.K., Han, Y.M., Peng, Z.X., Li, Y.F., 2016. Phthalate esters in indoor window films in a Northeastern Chinese Urban center: film growth and implications for human exposure. Environ. Sci. Technol. 50, 7743–7751.

Husain, Q., Qayyum, S., 2013. Biological and enzymatic treatment of bisphenol A and other endocrine disrupting compounds: a review. Crit. Rev. Biotechnol. 33, 260–292.

Inoue, D., Sawada, K., Sei, K., Ike, M., 2016. Detection of retinoic acid receptor antagonist contamination in the aquatic environment of the Kinki region of Japan. Water Res. 103, 58–65.

Iqbal, M., Nisar, J., Adil, M., Abbas, M., Riaz, M., Tahir, M.A., Younus, M., Shahid, M., 2017. Mutagenicity and cytotoxicity evaluation of photo-catalytically treated petroleum refinery wastewater using an array of bioassays. Chemosphere 168, 590–598.

Jin, H.B., Zhu, L.Y., 2016. Occurrence and partitioning of bisphenol analogues in water and sediment from liaohe river basin and Taihu lake, China. Water Res. 103, 343–351.

Johnson, C.M., Pleshko, N., Achary, M., Suri, R.P.S., 2014. Rapid and sensitive screening of 17 beta-estradiol estrogenicity using Fourier Transform infrared imaging spectroscopy (FT-IRIS). Environ. Sci. Technol. 48, 4581–4587.

Kalmykova, Y., Bjorklund, K., Stromvall, A.M., Blom, L., 2013. Partitioning of polycyclic aromatic hydrocarbons, alkylphenols, bisphenol A and phthalates in landfill leachates and stormwater. Water Res. 47, 1317–1328.

Kalmykova, Y., Moona, N., Stromvall, A.M., Bjorklund, K., 2014. Sorption and degradation of petroleum hydrocarbons, polycyclic aromatic hydrocarbons, alkylphenols, bisphenol A and phthalates in landfill leachate using sand, activated carbon and peat filters. Water Res. 56, 246–257.

Kosutic, K., Dolar, D., Strmecky, T., 2015. Treatment of landfill leachate by membrane processes of nanofiltration and reverse osmosis. Desalin. Water Treat. 55, 2680–2689.

Krivoshiev, B.V., Dardenne, F., Covaci, A., Blust, R., Husson, S.J., 2016. Assessing in-vitro estrogenic effects of currently-used flame retardants. Toxicol. Vitro 33, 153–162.

Kuch, B., Kern, F., Metzger, J.W., von der Trenck, K.T., 2010. Effect-related monitoring: estrogen-like substances in groundwater. Environ. Sci. Pollut. Res. 17, 250–260.

Lewis, S.E., Yokofich, A., Mohr, M., Kurth, C., Giuliani, R., Baldridge, M.G., 2012. Exposure to bisphenol A modulates hormone concentrations in Gammarus pseudolimnaeus. Can. J. Zool. 90, 1414–1421.

Li, J.L., Song, J., Bi, S., Zhou, S., Cui, J.W., Liu, J.G., Wu, D.M., 2016a. Electrochemical estrogen screen method based on the electrochemical behavior of MCF-7 cells. J. Hazard. Mater. 313, 238–243.

Li, J.Y., Zhao, L., Qin, L.L., Tian, X.J., Wang, A.M., Zhou, Y.M., Meng, L., Chen, Y., 2016b. Removal of refractory organics in nanofiltration concentrates of municipal solid waste leachate treatment plants by combined Fenton oxidative-coagulation with photo - Fenton processes. Chemosphere 146, 442–449.

Li, M., Xu, B., Liungai, Z.Q., Hu, H.Y., Chen, C., Qiao, J., Lu, Y., 2016c. The removal of estrogenic activity with UV/chlorine technology and identification of novel estrogenic disinfection by-products. J. Hazard. Mater 307, 119–126.

Li, P., Mei, Z., Cai, W.S., Shao, X.G., 2014. Rapid analysis of phthalic acid esters in environmental water using fast elution gas chromatography with mass spectrometry and adaptive library spectra. J. Sep. Sci. 37, 1585–1590.

Li, Z.Q., Zhu, Q., Chen, H., Hu, L.Y., Negi, H., Zheng, Y., Ahmed, Y., Wu, Z.H., Li, D.W., 2016d. Binding of anterior gradient 2 and estrogen receptor-alpha: dual critical roles in enhancing fulvestrant resistance and IGF-1-induced tumorigenesis of breast cancer. Cancer Lett. 377, 32–43.

Loffredo, E., Castellana, G., 2015. Comparative evaluation of the efficiency of low-cost adsorbents and ligninolytic fungi to remove a combination of xenoestrogens and pesticides from a landfill leachate and abate its phytotoxicity. Environ. Eng. 50, 958–970.

Long, Y.Y., Xu, J., Shen, D.S., Du, Y., Feng, H.J., 2017. Effective removal of contaminants in landfill leachate membrane concentrates by coagulation. Chemosphere 167, 512–519.

Ma, T.T., Chen, L.K., Wu, L.H., Zhang, H.B., Luo, Y.M., 2016. Oxidative stress, cytotoxicity and genotoxicity in earthworm Eisenia fetida at different di-n-butyl phthalate exposure levels. Plos One 11.

Molins-Delgado, D., Diaz-Cruz, M.S., Barcelo, D., 2016. Ecological risk assessment associated to the removal of endocrine-disrupting parabens and benzophenone-4 in wastewater treatment. J. Hazard. Mater. 310, 143–151.

Nwosu, U.G., Cook, R.L., 2015. C-13 nuclear magnetic resonance and electron paramagnetic spectroscopic comparison of hydrophobic acid, Transphilic acid, and reverse osmosis may 2012 isolates of organic matter from the suwannee river. Environ. Eng. Sci. 32, 14–22.

Ogunlaja, O.O., Parker, W.J., 2015. Assessment of the removal of estrogenicity in biological nutrient removal wastewater treatment processes. Sci. Total Environ. 514, 202–210.

Olmez-Hanci, T., Dursun, D., Aydin, E., Arslan-Alaton, I., Girit, B., Mita, L., Diano, N., Mita, D.G., Guida, M., 2015. S2O82-/UV-C and H2O2/UV-C treatment of Bisphenol A: assessment of toxicity, estrogenic activity, degradation products and results in real water. Chemosphere 119, S115–S123.

Oturan, N., van Hullebusch, E.D., Zhang, H., Mazeas, L., Budzinski, H., Le Menach, K., Oturan, M.A., 2015. Occurrence and removal of organic micropollutants in landfill leachates treated by electrochemical advanced oxidation processes. Environ. Sci. Technol. 49, 12187–12196.

Oulego, P., Collado, S., Laca, A., Diaz, M., 2016. Impact of leachate composition on the advanced oxidation treatment. Water Res. 88, 389–402.

Panagiotakis, I., Dermatas, D., Vatseris, C., Chrysochoou, M., Papassiopi, N., Xenidis, A., Vaxevanidou, K., 2015. Forensic investigation of a chromium(VI) groundwater plume in Thiva, Greece. J. Hazard. Mater. 281, 27–34.

Papa, M., Ceretti, E., Viola, G.C.V., Feretti, D., Zerbini, I., Mazzoleni, G., Steimberg, N., Pedrazzani, R., Bertanza, G., 2016. The assessment of WWTP performance: towards a jigsaw puzzle evaluation. Chemosphere 145, 291–300.

Paskuliakova, A., Tonry, S., Touzet, N., 2016. Phycoremediation of landfill leachate with chlorophytes: phosphate a limiting factor on ammonia nitrogen removal. Water Res. 99, 180–187.

Ren, G.B., Zhou, M.H., Liu, M.M., Ma, L., Yang, H.J., 2016. A novel vertical-flow electro-Fenton reactor for organic wastewater treatment. Chem. Eng. J 298, 55–67.

Silva, T., Silva, M.E.F., Cunha-Queda, A.C., Fonseca, A., Saraiva, I., Sousa, M.A., Goncalves, C., Alpendurada, M.F., Boaventura, R.A.R., Vilar, V.J.P., 2013. Multi-stage treatment system for raw leachate from sanitary landfill combining biological nitrification denitrification/solar photo-Fenton/biological processes, at a scale close to industrial Biodegradability enhancement and evolution profile of trace pollutants. Water Res. 47, 6167–6186.

Silva, T., Vieira, E., Lopes, A.R., Nunes, O.C., Fonseca, A., Saraiva, I., Boaventura, R.A.R., Vilar, V.J.P., 2017. How the performance of a biological pre-oxidation step can affect a downstream photo-Fenton process on the remediation of mature landfill leachates: assessment of kinetic parameters and characterization of the bacterial communities. Sep. Purif. Technol. 175, 274–286.

Suzuki, T., Yaguchi, K., Suzuki, S., Suga, T., 2001. Monitoring of phthalic acid monoesters in river water by solid-phase extraction and GC-MS determination. Environ. Sci. Technol. 35, 3757–3763.

Svenson, A., Sjoholm, S., Allard, A.S., Kaj, L., 2011. Antiestrogenicity and estrogenicity in leachates from solid waste deposits. Environ. Toxicol. 26, 233–239.

Talalaj, I.A., 2015. Mineral and organic compounds in leachate from landfill with concentrate recirculation. Environ. Sci. Pollut. Res. 22, 2622–2633.

Umar, M., Aziz, H.A., Yusoff, M.S., 2010. Trends in the use of Fenton, electro-Fenton and photo-Fenton for the treatment of landfill leachate. Waste Manage 30, 2113–2121.

Vantangoli, M.M., Madnick, S.J., Wilson, S., Boekelheide, K., 2016. Estradiol exposure differentially alters monolayer versus microtissue MCF-7 human breast carcinoma cultures. Plos One 11.

Wang, G., Lu, G., Yin, P., Zhao, L., Yu, Q.J., 2016a. Genotoxicity assessment of membrane concentrates of landfill leachate treated with Fenton reagent and UV-Fenton reagent using human hepatoma cell line. J. Hazard. Mater. 307, 154–162.

Wang, G.F., Lu, G., Zhao, J.D., Yin, P.H., Zhao, L., 2016b. Evaluation of toxicity and estrogenicity of the landfill-concentrated leachate during advanced oxidation treatment: chemical analyses and bioanalytical tools. Environ. Sci. Pollut. Res. 23, 16015–16024.

Wu, P.G., Cai, C.G., Yang, D.J., Wang, L.Y., Zhou, Y., Shen, X.H., Ma, B.J., Tang, J., 2015. Identification of 19 phthalic acid esters in dairy products by gas chromatography with mass spectrometry. J. Sep. Sci. 38, 254–259.

Xing, W., Lu, W.J., Zhao, Y., Zhang, X., Deng, W.J., Christensen, T.H., 2013. Environmental impact assessment of leachate recirculation in landfill of municipal solid waste by comparing with evaporation and discharge (EASEWASTE). Waste Manage 33, 382–389.

Xu, Z.W., Zhang, W.M., Lv, L., Pan, B.C., Lan, P., Zhang, Q.X., 2010. A new approach to catalytic degradation of dimethyl phthlate by a macroporous OH-Type strongly basic anion exchange resin. Environ. Sci. Technol. 44, 3130–3135.

Yiu, K.W., Lee, C.K., Kwok, K.C., Cheung, N.H., 2014. Measuring the kinetics of the binding of xenoestrogens and estrogen receptor alpha by Fluorescence polarization. Environ. Sci. Technol. 48, 11591–11599.

Zhang, H., Chang, C.H., Lu, F., Lee, D.J., He, P.J., Shao, L.M., Su, A., 2009. Estrogenic activity of fractionate landfill leacahte. Sci. Total Environ. 407, 879–886.