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ORIGINAL ARTICLES

Fenton Oxidation of Bisphenol A using an Fe_3O_4-coated Carbon Nanotube: Understanding of Oxidation Products, Toxicity and Estrogenic Activity

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Abstract This study focused on Fenton oxidation of bisphenol A using a Fe_3O_4-coated multi-walled carbon nanotube (Fe_3O_4/MWCNT), from which oxidation products, acute toxicity and estrogenic activity were assessed. The final effluents showed no biological toxicity to 24 h born *Daphnia magna* and negligible *in-vivo* estrogenic activity. The major oxidation productions generated from heterogeneous Fenton oxidation of bisphenol A included 4-hydroxyacetophenone, 4-hydroxybenzoic acid, catechol and hydroquinone. Therefore, Fenton oxidation of bisphenol A using Fe_3O_4/MWCNT with a low dose of H_2O_2 led to high removal of bisphenol A with negligible aquatic toxicity and estrogenic activity. It would be a cost-effective solution for treatment and reuse of emerging contaminant-containing wastewater and water.

Key words Biotoxicity, Estrogenic activity, Bisphenol A, Carbon nanotube, Fe_3O_4, Advanced oxidation

Introduction

Bisphenol A (BPA), as a well-known endocrine disrupting compound, has been widely used in various industries for past decades (Staples et al., 1998; Rivas et al., 2009; Mboula et al., 2013). Although BPA has been detected at trace levels in wastewater, surface water, landfill leachate and drinking water (Suzuki et al., 2004; Liu et al., 2009; Mohapatra et al., 2010; Xiao et al., 2012), BPA at dilute concentrations can cause hormonal disruption and various cancers (Alexander et al., 1988; Ike et al., 2002; Kuruto-Niwa et al., 2002; Gultekin and Ince 2007; Kim et al., 2007; Pant and Deshpande 2012; Xiao et al., 2012). Thus, effective remediation technologies need to be developed to eliminate BPA from wastewater and water.

Various methods including biodegradation, advanced oxidation and adsorption have been studied for removal of BPA from various wastewater and water (Torres-Palma *et al.*, 2007; Mohapatra et al., 2010; Park et al., 2010; Torres-Palma et al., 2010). Fenton oxidation using H_2O_2 and iron catalysts among various methods was found to be a highly efficient and cost-effective method for treating BPA (Katsumata et al., 2004; Ioan et al., 2007). However, homogenous Fenton oxidation have the limitations such as low efficiency at neutral pH and generation of excessive iron sludge after reactions (Catrinescu *et al.*, 2003; Pignatello et al., 2006; Diya'uddeen et al., 2012). In order to enhance efficiency of the homogeneous Fenton oxidation heterogeneous Fenton oxidation have been developed by immobilization of iron oxides and catalysts onto various carbon supports (Walling, 1975; Valentine and Wang 1998; Feng et al., 2004; Hsueh et al., 2006; Lim 2006; Oliveira et al., 2007; Hanna et al., 2008).

Recently we prepared the Fe_3O_4-coated multi-walled carbon nanotube (Fe_3O_4/MWCNT, hereafter) for effective

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Fenton oxidation of BPA in water (Cleveland et al., 2014). The Fenton oxidation of BPA using Fe_3O_4/MWCNT has demonstrated high removal of BPA and COD (i.e., 97-100 % for BPA, 8-60% for Chemical Oxygen Demand) with the [H_2O_2] : [BPA] of 4 to 10 (mol H_2O_2/mol BPA) (Cleveland et al., 2014).

However, acute toxicity and estrogenic activity of BPA oxidation products in the treated BPA solution have not been investigated despite these activities are very important for safe and effective treatment, and reuse of treated water. To the best of our knowledge, no studies have been done to assess the aquatic toxicity and the estrogenic activity of BPA oxidation products generated by the Fe_3O_4/MWCNT-driven heterogeneous Fenton oxidation.

Therefore, the objective of the present study was to assess acute toxicity and estrogenic activity of BPA oxidation products generated by the Fe_3O_4/MWCNT-driven heterogeneous Fenton oxidation. Specifically, the acute toxicity using *Daphnia magna* and in-vivo endogenic activity of BPA oxidation products from the heterogeneous Fenton oxidation of BPA were evaluated. The oxidation products were also identified.

Material and Methods

Chemicals and reagents

Multi-walled carbon nanotubes (MWCNT, inside diameter of 5-10 nm and an outside diameters of 60-100 nm with a length of 0.5-500 μm, surface area of 40-300 m^2/g) (Kumar and Mohan 2012), BPA, ferrous sulfate ($FeSO_4$) and hydrogen peroxide were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Acute toxicity of BPA oxidation products

The Fe_3O_4/MWCNT was prepared by the chemical oxidation followed by co-precipitation as described by Cleveland et al. (2014). The Fenton oxidation was conducted in a glass flask containing 100 mL DI water with 70 mg L^{-1} (0.31 mM) BPA at the selected conditions (molar ratio of [H_2O_2] : [BPA] of 4, pH 3 and 50 mg Fe_3O_4/MWCNT). The pH of aqueous solution for the Fenton oxidation was adjusted with 1 N NaOH and 1 N HCl. These conditions were chosen as the optimum conditions as reported by Cleveland et al. (2014). Although heterogeneous Fenton oxidation would occur at broad ranges of pH, our previous study (Cleveland *et al.*, 2014) indicated highest BPA removal efficiencies at pH 3 which was used for this study. Besides, there was negligible leaching of iron from the Fe_3O_4 in the carbon nanotube during the Fenton oxidation because of low concentration of H_2O_2 during for the Fenton oxidation.

The final effluent after 4 h reaction was used for the toxicity tests after the pH of final effluent was adjusted to pH 7. The toxicity of BPA and its oxidation products in the final effluent generated by the Fenton oxidation at the above conditions was tested using 24 h born *Daphnia magna* at various dilutions (Chahbane et al., 2014). This protocol was based on the procedure as Organization for Economic Cooperation and Development (OECD) Guideline 202. The acute toxicity tests were conducted with five *Daphnia magna* in each glass flask containing 50 mL of reaction solution with various dilution ratios. Then the *Daphnia magna* species were cultivated with the final effluent in an incubator at 20ºC in a light/dark cycle (16 h/8 h) for 24-48 h. The toxicity was determined as immobilization % obtained by counting the number of survived *Daphnia magna* after 24 and 48 h.

In-vivo endogenic activity of BPA oxidation products

The final effluent generated by the Fenton oxidation which was used for the biotoxicity test was also used for the *in-vivo* estrogenic activity of BPA oxidation products. To measure the estrogenic activity of BPA oxidation products, the MCF7-BUS cell line (estrogen-sensitive human breast cancer cells), was kindly provided by Dr. Ana Soto (Tufts University, Boston, MA, USA). The cells were grown in Dulbecco's Modified Eagle Medium (DMEM; Gibco BRL, Grand Island, NY, USA) supplemented with 5% fetal bovine serum (FBS; Hyclone, Logan, UT, USA), penicillin (100 units/ml) and streptomycin (100 μg/ml) at 37ºC in an atmosphere of 5% CO_2/95% air under saturating humidity. The E-screen assay using the MCF7-BUS cells was conducted to identify the estrogenic activity of the BPA solution without Fenton oxidation (70 mg/L) and the BPA solution treated by Fe_3O_4/MWCNT-driven Fenton oxidation at the conditions (molar ratio of [H_2O_2] : [BPA] of 4, pH 3 and 50 mg Fe_3O_4/MWCNT). Charcoal dextran-treated FBS (CDFBS) was prepared as described (Min et al., 2012) and stored at -20ºC. The cells were harvested with 0.05% trypsin-0.53 mM EDTA·4Na and resuspended in 5%-FBS DMEM. They were seeded onto 48-well plate at a density of 5×10^3 cells/well and incubated for 48 hours. The cells were then treated with 10%-CDFBS DMEM containing three doses of sample A and B (6, 60 and 600 nM) in triplicate. After incubating the cells for 6 days, sulforhodamine B assay was carried out to evaluate the extent of cell proliferation. The relative proliferation effect (RPE) was calculated using the following equation.

$$RPE = [(S - 1) / (E - 1)] \times 100$$

where S = proliferation of the samples and E = proliferation of positive control (10-10 M E2)

The results of each assay were expressed as mean ± standard deviation. Differences between groups were assessed by one-way ANOVA followed by Duncan's post-hoc test. Statistical significance was accepted at $p < 0.01$.

Identification and quantification of BPA and its oxidation products

Chromatographic separation was performed with an AB Sciex triple quadrupole 450 LC-Q-MS/MSD (AB Sciex C0., USA) equipped with a binary pump, a vacuum degasser, low carryover autosampler, a thermostatted column compartment and a Mass Hunter data system. Ten microliters of the extract were injected onto a Kinetex C18 column (100 × 2.1 mm, 3 μm, Phenomenex Co., USA). The mobile phases A and B consisted of 2 mM ammonium acetate in water and methanol, respectively. The analysis for BPA, 4-hydroxyacetophenone (4-HAP), catechol and hydroquinone was performed by using the following gradient program: at a flow rate of 700 μL/min the gradient was initially started at 33% methanol and maintained at that level for 4 min. The gradient was then increased to 60% methanol between 4 and 6 min, increased to 95% methanol between 6 and 7 min, decreased to 33% methanol between 7.1 and 9.5 min, and maintained at this percentage between 9.5 and 12 min.

Results and discussion

The TEM and SEM images of the Fe_3O_4/MWCNT showed deposition of the Fe_3O_4 particles (100-150 nm) onto the carbon nanotube (Fig. 1). The Fe_3O_4 in the Fe_3O_4/MWCNT has an octahedron crystal structure. It also indicates that the Fe_3O_4 is well deposited on the MWCNT with little aggregation. The XRD patterns of the Fe_3O_4/MWCNT also present the major components in the Fe_3O_4/MWCNT is Fe_3O_4 (Cleveland et al., 2014).

The heterogeneous Fenton oxidation of BPA using the Fe_3O_4/MWCNT at the selected conditions (70 mg L^{-1} BPA, 50 mg Fe_3O_4/MWCNT, mol H_2O_2/mol BPA of 4, 20°C, pH 3) was conducted for analyzing the toxicity and estrogenic activity of BPA and its oxidation products. Excellent recoveries (72.5~107.3.4%) were obtained for BPA and its oxidation products such as 4-hydroxyacetophenone (4-HAP), 4-hydroxybenzoic acid (4-HBA), catechol and hydroquinone from the control wastewater (Table 1). The final effluents from the Fenton oxidation of BPA at the above conditions revealed that BPA, 4-HAP, 4-HBA, catechol and hydroquinone were detected at the level of 0.05, 0.06, 0.03, < 0.05 and < 0.05 ng/g in the treated waste water (Table 2). The results in Table

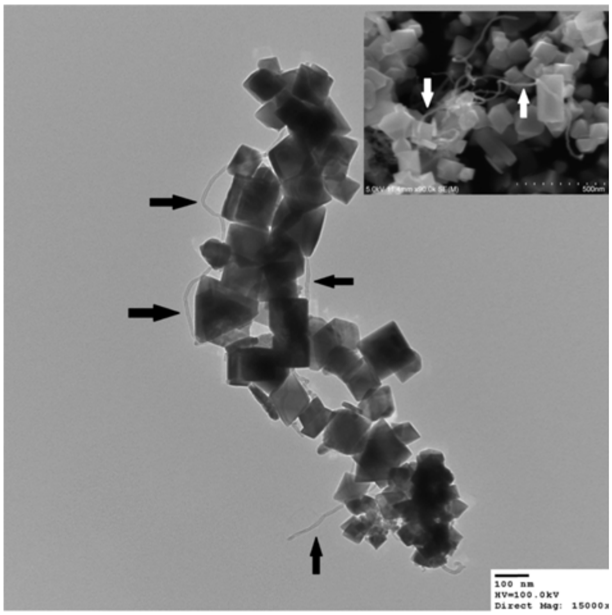

Fig. 1. TEM and SEM (inserts) images of the Fe_3O_4/MWCNT (Cleveland et al., 2014). The arrows indicate MWCNTs.

Table 1. Recovery of BPA, 4-HAP[1], 4-HBA[2] in synthetic wastewater

Compounds	Fortification (0.5 μg/g)	% recovered ± standard deviation (SD)				[3]LOD (ng/g)
		1	2	3	Average	
BPA	Water	107.3	94.1	90.2	97.2 ± 9.0	0.01
4-HAP	Water	83.9	86.2	78.3	82.8 ± 4.1	0.01
4-HBA	Water	72.5	76.3	82.9	77.2 ± 5.3	0.01

[1]HAP: 4-hydroxyacetophenone.
[2]HBA: 4-hydroxybenzoic acid.
[3]LOD: Limit of Detection.

Table 2. Concentrations of BPA and its oxidation products in the treated wastewater

Treated wastewater	Concentration (ng/g) ± standard deviation (SD)				
	BPA	4-HAP[1]	4-HBA[2]	Catechol	Hydroquinone
	0.05 ± 0.01	0.06 ± 0.01	0.03 ± 0.01	< 0.05	< 0.05

[1]HAP: 4-hydroxyacetophenone.
[2]HBA: 4-hydroxybenzoic acid.

Table 3. Cumulative immobility of *Daphnia* for biotoxicity measurement.

Test group	*Daphnia* (ea)	Cumulative immobility	
		24 h	48 h
Control	20	0	0
Waste water	20	0	0

2 are consistent with those from the Fenton oxidation pathways of BPA reported by Poerschmann et al. (2010), Olmez-Hanci et al. (2015), and Hua et al. (2014). Particularly the Fenton oxidation of BPA (i.e., [H_2O_2]/[BPA] of 2 to 4) made at similar conditions used for the present study, as Poerschmann et al. (2010) reported, indicated that the major oxidation products were 4-HAP, catechol and hydroquinone.

The acute biotoxicity tests using 24 h born *Daphnia magna* were conducted to estimate aquatic toxicity of the BPA oxidation products in the BPA solution treated by the Fenton oxidation using Fe_3O_4/MWCNT at the selected conditions (70 mg L^{-1} BPA, 50 mg Fe_3O_4/MWCNT, mol H_2O_2/mol BPA of 4, 20°C, pH 3) (Table 3). The results showed that any immobility of *Daphnia magna* were not observed from the negative control (the DI water as a sample) and the BPA solution treated by Fenton oxidation using Fe_3O_4/MWCNT after 24 h and 48 h incubation (Table 3). This supported no biological toxicity of the BPA intermediates and products in the treated BPA solution at the selected conditions since the oxidation products of BPA stayed at 0.03-0.06 mg/L in the aqueous solution. These results are similar to our previous investigation to demonstrate negligible aquatic toxicity in the BPA solution treated by Fe_3O_4/MWCNT-driven Fenton oxidation using Toxi-Chromotest™ (Environmental Biodetection Products Inc., Mississauga, Canada) (Cleveland *et al*., 2014). The assessment of biotoxicity using Toxi-Chromotest™ relied on the measurement of toxicant-based inhibition of β-galactosidase synthesis by *E. coli* as described in by Schalie et al. (2006). Therefore, the Fenton oxidation of BPA using the Fe_3O_4/MWCNT led to the effective treatment of BPA in wastewater and water. It would contribute to protection of various water resources and helping reuse of wastewater for agricultural and industrial application.

The *in-vivo* estrogenic activity tests also showed negligible activities from the BPA solution treated by Fe_3O_4/MWCNT-driven Fenton oxidation at the selected conditions (70 mg L^{-1} BPA, 50 mg Fe_3O_4/MWCNT, mol H_2O_2/mol BPA of 4, 20°C, pH 3) as seen in Fig. 2. The proliferative effects of the BPA solution without Fenton oxidation (70 mg L^{-1}) and the BPA solution treated by Fe_3O_4/MWCNT-driven Fenton oxidation on MCF7-BUS cells were examined for determining their estrogenic activities. The effects of compounds relative to E2 (10-10 M), which showed the maximum cell proliferative effects, represented as RPE. The frequency of proliferation increased dose-dependently in the BPA solution (untreated by the Fenton oxidation)-treated MCF7-BUS cells, whereas it did not occur in the BPA solution (degraded by the Fenton oxidation)-treated cells (Fig. 2). These results in Fig. 2 supported that the heterogeneous Fenton oxidation of BPA using the Fe_3O_4/MWCNT resulted in the negligible estrogenic activity. There were a few of publication to report the estrogenic activity of BPA solution treated by advanced oxidation techniques including Fenton oxidation and photochemical oxidation. As reported by Olmez-Hanci et al. (2015), H_2O_2/ultraviolet light (UV-C) and $S_2O_8^{2-}$/ultraviolet light (UV-C) processes with high dose of H_2O_2 showed the complete elimination of estrogenic activity originally retained in the BPA solution (20 mg L^{-1}) when the estrogenic activity was evaluated using the Yeast Estrogen Screen method. On the contrary, the untreated BPA solution and UV-C treated BPA solution did present the clear estrogenic activity (Olmez-Hanci et al., 2015). Compared with the estrogenic results using the above methods (UV-C, H_2O_2/UV-C and $S_2O_8^{2-}$/UV-C) requiring high energy consumption and chemical dose the heterogeneous Fenton oxidation of BPA at low dose of H_2O_2 in this study clearly benefit cost effective and safe remediation while almost completely eliminating biotoxicity and estrogenic activity.

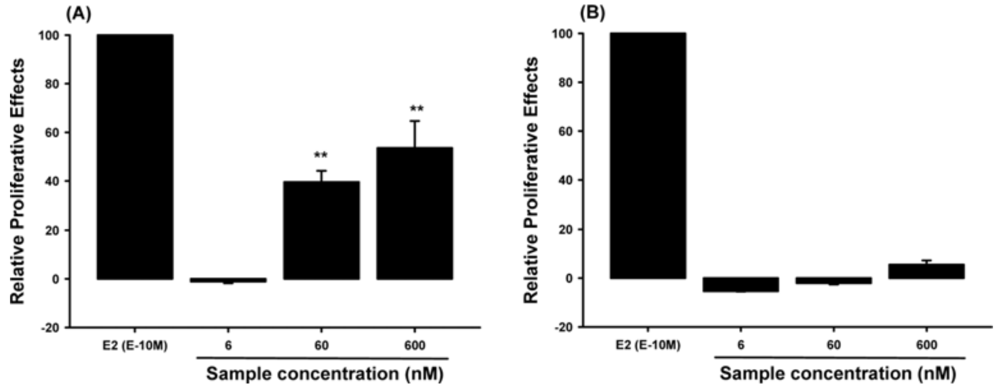

Fig. 2. Effects of BPA solution without Fenton oxidation (70 mg/L, A) and BPA solution treated by the MWCNT/ Fe_3O_4-driven Fenton oxidation (B) on the MCF7-BUS cell proliferation. E2 indicates 17β-estradiol (E2, estrogen) as a positive control. The negative control (the buffer solution) did not show any estrogenic activity. The results of each assay are expressed as mean ± standard deviation (one-way ANOVA followed by Duncan's post-hoc test. $^{**}p < 0.01$).

In conclusion, the oxidation products, acute toxicity and estrogenic activity for heterogeneous Fenton oxidation of bisphenol A using Fe_3O_4/MWCNTs were assessed when the Fenton oxidation occurred at the selected conditions (70 mg L^{-1} BPA, 50 mg Fe_3O_4/MWCNT, mol H_2O_2/mol BPA of 4, 20°C, pH 3). The final effluents from the Fenton oxidation of BPA at the selected conditions showed no biological toxicity when it was tested with 24 h born *Daphnia magna*. It also resulted in negligible estrogenic activity when in-vivo estrogenic activity of the BPA oxidation products was evaluated. The major oxidation productions generated from the Fenton oxidation of BPA were 4-hydroxyacetophenone (4-HAP), 4-hydroxybenzoic acid (4-HBA), catechol and hydroquinone Therefore, the Fenton oxidation of bisphenol A using Fe_3O_4/ MWCNT with low dose of H_2O_2 led to high removal of BPA with negligible biotoxicity and estrogenic activity.

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