Supporting Information for

Transformation of Bisphenol A and Alkylphenols by Ammonia-oxidizing Bacteria through Nitration

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For Environmental Science and Technology

11 pages, including 6 figures.

Page 2 through 4 provide details on the chemicals and analytical methods.

Page 5 includes Table S1-S2, which are referred to directly in the Experimental and Results portion of the text.

Pages 6 through 11 include Figures S1-S6, which are referred to directly in the

Experimental, Results and Discussion portion of the text.

EXPERIMENTAL

Synthesis of dinitro-BPA

The nitration step was conducted by treating 100 mg/L BPA with excessive $NaNO_2$ (6250 mg N/L) in phosphate buffer at pH 6.0 with the reaction temperature of 30 ^{o}C. After 36 h synthesis, dinitro-BPA was precipitate as powder in dark brown. Dinitro-BPA was separated by filtering 250 mL reaction solution through a glass microfiber filter (GF/C Whatman, USA), and further dried and eluted by ether. The complete conversion of BPA to dinitro-BPA and the purity of recovered dinitro-BPA were confirmed by HPLC. In addition, HPLC-QqQ MS analysis found no MS response for BPA (227>133, 227>211) and nitro-BPA (272>240, 272>227) under the highest dinitro-BPA concentration (25 μg/L) of the calibration curve.

Growth of *Nitrosomonas europaea*.

N. europaea was grown in a defined mineral salt medium containing 25 mM of $(NH_4)_2SO_4$ (corresponding to 700 mg-N/L), 43 mM KH_2PO_4, 750 μM $MgSO_4$, 200 μM $CaCl_2$, 10 μM $FeSO_4$, 16.5 μM EDTA, 0.5 μM $CuSO_4$, 4 mM NaH_2PO_4, and 3.8 mM Na_2CO_3. The final pH of the medium was adjusted close to 8.0 by NaOH. The cells were grown in 2000 mL glass flasks containing 1000 mL of growth medium. The flasks were incubated in the dark at 150 rpm, at 30 °C for 5 days before harvesting for

degradation studies. The cells were pelleted by centrifugation (at 10000×g and 4 ^{o}C for 30 min), and then washed once with phosphate buffer solution (50 mM NaH_2PO_4 [pH 7.8], and 2 mM $MgSO_4 \cdot 7H_2O$). The washed cell pellets were resuspended in the *N. europaea* growth medium.

Yeast estrogenic screening (YES) assay

The YES assay is an in vitro, yeast-based reporter assay and a recombinant yeast *Saccharomyces cerevisiae* strain, carrying a human estrogen receptor (ERα) and a reporter gene (*Lac Z*), was used.[23] The YES assays were conducted as follows. Ether extracts were dried and resuspended in proper amount of dimethyl sulfide (DMSO) for YES assay. 2 μL of 17β-estradiol (E2, 1 μM to 1.4 nM, 3-fold serial dilutions), DMSO (negative control), and samples were mixed with 198 μL of assay medium containing the cell-suspension of recombinant *S. cerevisiae* strain and chlorophenol red-β-D galactopyranoside in 96-well microplates, and incubated at 30 °C for 3 days. E2, DMSO, and samples were all tested in triplicate.

Solid phase extraction

The C18 solid phase extraction (SPE) cartridge (CNW, Shanghai Anpel Scientific Instrument, China) were conditioned with 5 mL of acetic ether, 5 mL of methanol and 10 mL of distilled water (pH at 2) at a flow rate of about 3 mL/min. Samples were then loaded through the cartridges at a flow rate of about 5 mL/min using a vacuum manifold system (Waters) connected to a vacuum pump. The loaded cartridges were

rinsed with 6 mL of a mixture methanol/water (5:95, *v:v*) and dried up for 30 min, then eluted with 6 mL of acetic ether at a flow rate of about 1 mL/min.

Analytical methods

Concentrations of nitrite and nitrate were measured by injecting 10 µL of liquid samples into an ion chromatography (IC) (Dionex ICS 3000, Dionex, USA). The pH was measured by a pH electrode (Denver Instrument, USA). The determination of ammonia was achieved by flow injection analysis (LACHAT-QC8500, Lachat instrument, USA). In brief, ammonia reacted with alkaline phenol, and then with sodium hypochlorite to form indophenols blue, where sodium nitroprusside was added to enhance sensitivity. The absorbance of the reaction product was measured at 630 nm.

TABLES

Table S1 MS parameters for the quantification of the analytes in the MRM mode

Analytes	Retention time (min)	Precursor ion (*m/z*)	Product ion (*m/z*)			DP (V)	EP (V)	CE (V)	CXP (V)
			1	2	3				
BPA	5.88	227	133	211	/	-70	-6	-32	-15
BPA-d16	5.92	241	142	223	/	-70	-6	-32	-15
Nitro-BPA	6.66	272	240	227	211	-64	-5	-38	-3
Dinitro-BPA	6.95	317	285	227	/	-64	-5	-38	-3

Table S2 Analytical results of different wastewater samples.

	NH_4^+-N (mg/L)	NO_2^--N (mg/L)	pH	DO (mg/L)	BPA （μg/L）	Dinitro-BPA （μg/L）
Influent	33.5	0.48	7.63	N.A.[a]	2.14	N.D.[b]
Beginning of oxidation ditch	32.9	0.08	7.66	0.19[c]	1.67	N.D.
End of oxidation ditch	1.13	0.34	7.66	0.94 [c]	0.71	0.0019
Final effluent	1.07	0.33	7.63	N.A.	0.50	0.0037

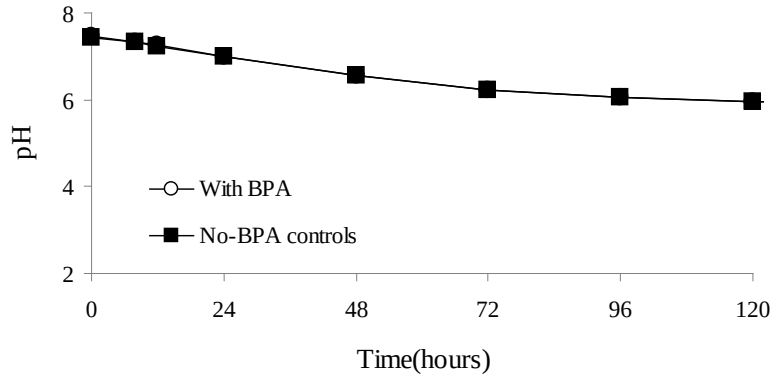
[a] Not available (not measured)

[b] Not detected

[c] Values were obtained from the WWTP online monitoring system

92 **FIGURES**

(a)



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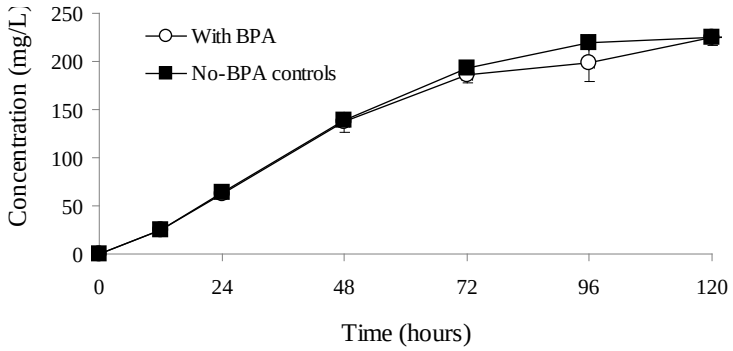
(b)



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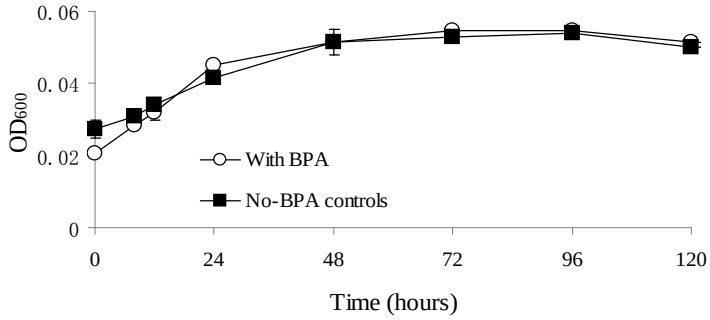
(c)



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96 Figure S1 The pH changes (a), nitrite production (b) and cell growth (c) during the

97 biodegradation of BPA by *N. europaea*.

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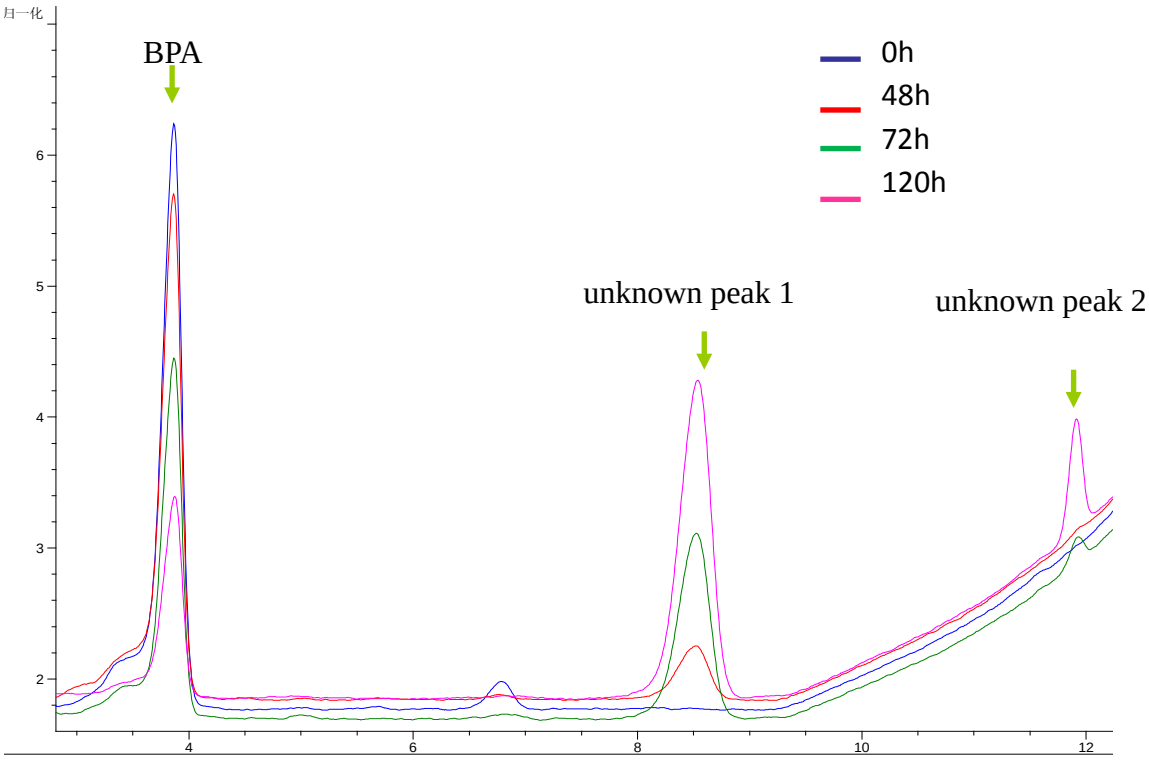
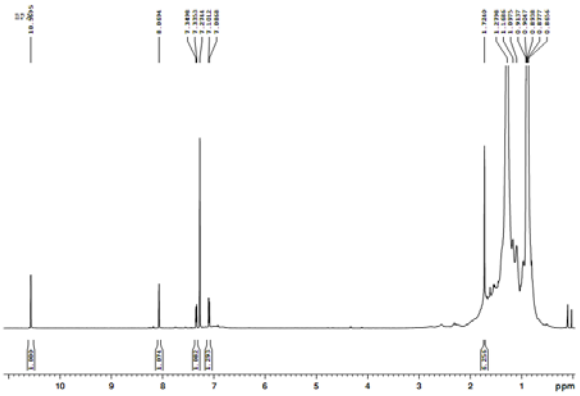
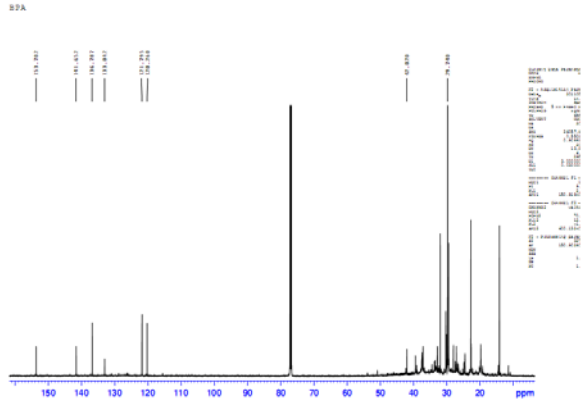


Figure S2 HPLC chromatograms during the biodegradation of BPA by *N. europaea*.

(a)



(b)



(c)

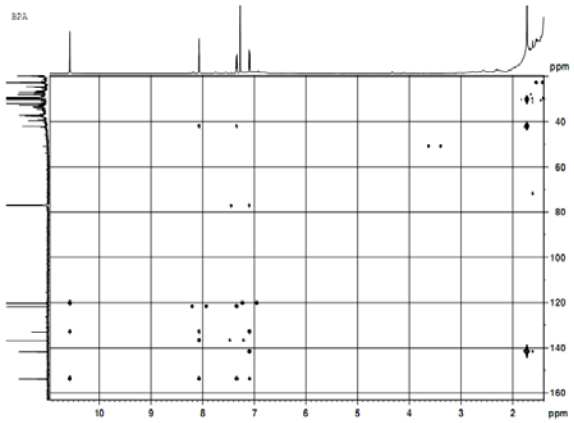


Figure S3 ^{1}H NMR (a), ^{13}C NMR (b) and HMBC NMR (c).

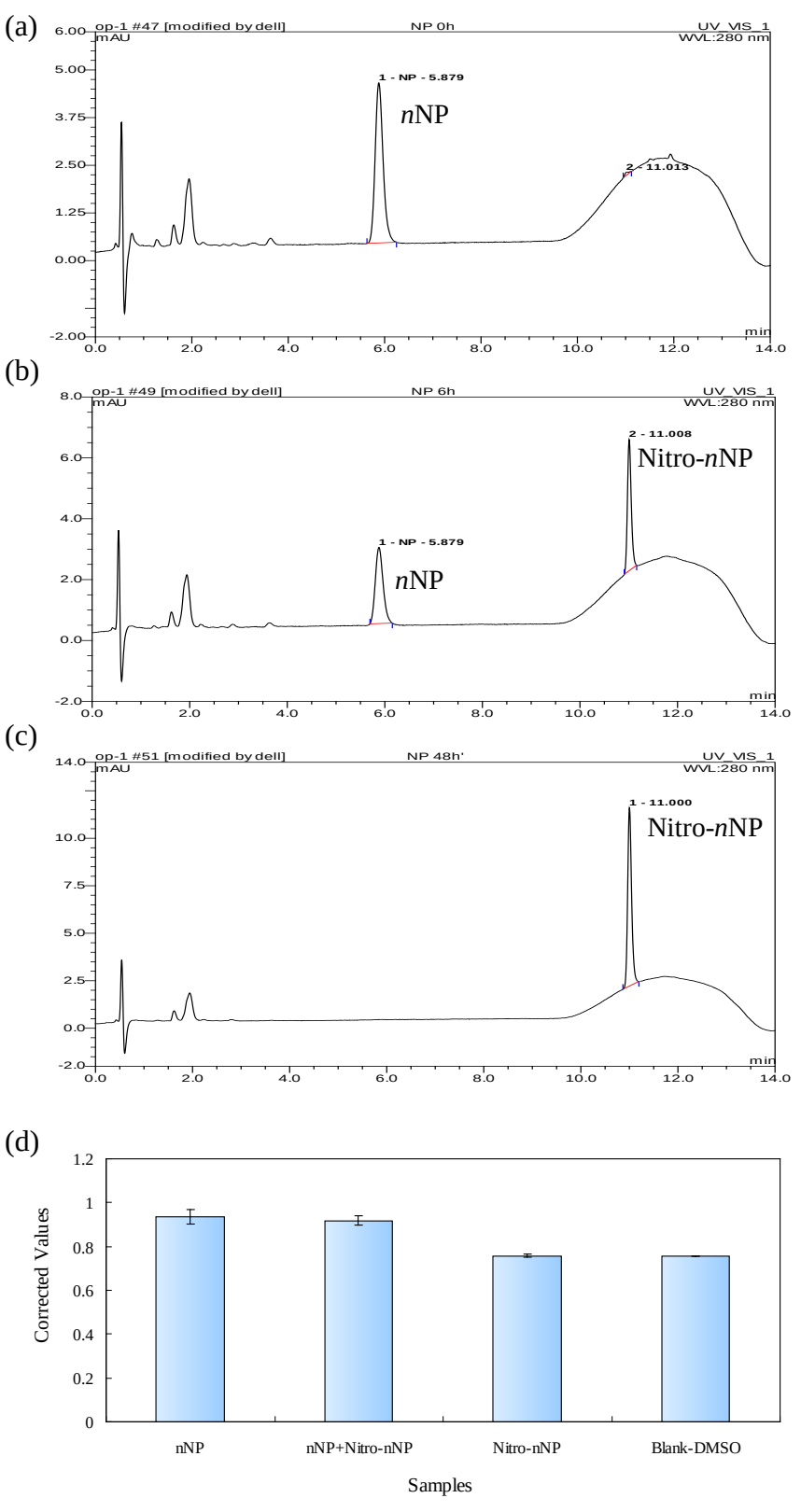


Figure S4 Chromatograms of *n*NP samples used for YES assays (a)-(c) and results from YES assays performed on the corresponding samples (d).

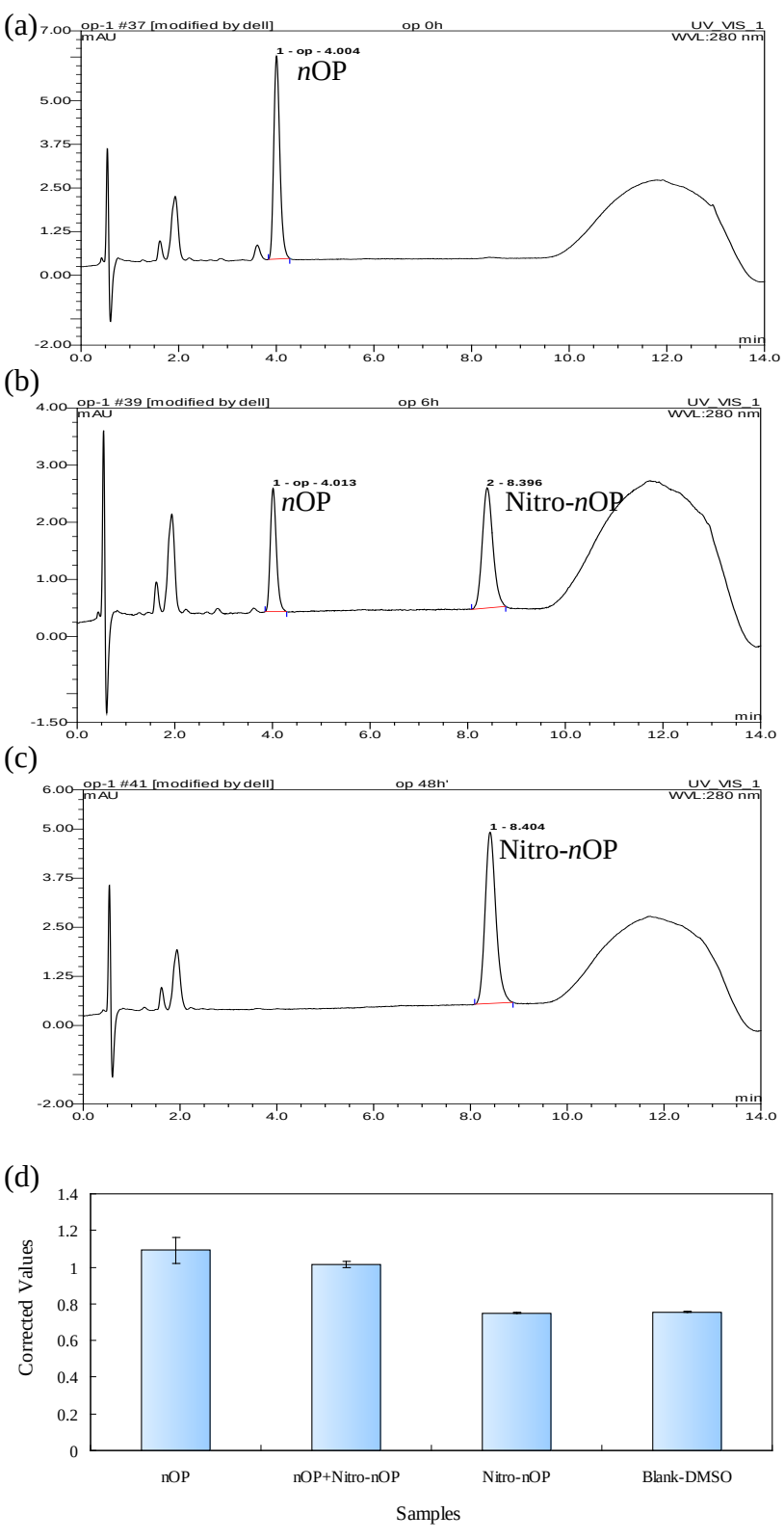


Figure S5 Chromatograms of *n*OP samples used for YES assays (a)-(c) and results from YES assays performed on the corresponding samples (d).

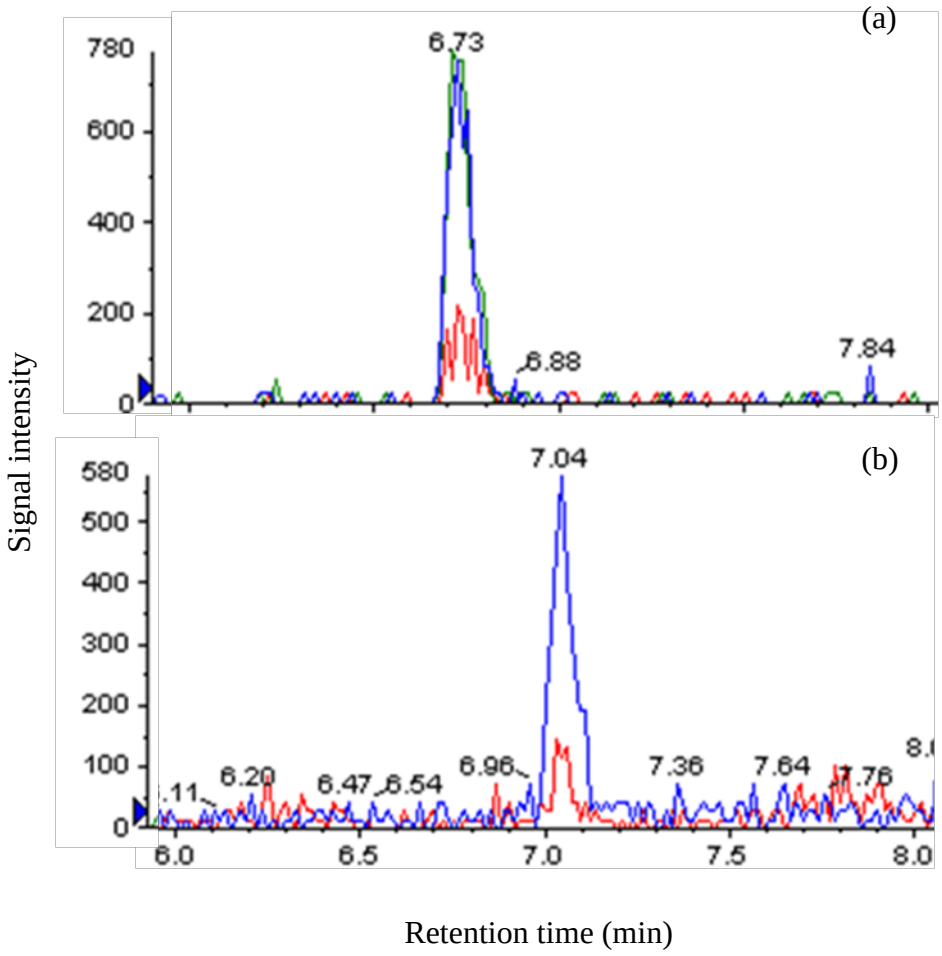


Figure S6 MRM chromatograms of nitro-BPA (a) and dinitro-BPA (b) of the oxidation ditch effluent water from Aug, 2011.