Supporting Information

Formation of Nitrophenolic Byproducts during Heat-Activated Peroxydisulfate Oxidation in the Presence of Natural Organic Matter and Nitrite

Peizeng Yang[†], Yuefei Ji[†], Junhe Lu[†*], Qingguo Huang[‡]

[†]Department of Environmental Science and Engineering, Nanjing Agricultural University, Nanjing 210095, China

[‡] Department of Crop and Soil Sciences, University of Georgia, Griffin, GA 30223, USA

[*]Corresponding author: e-mail: jhlu@njau.edu.cn

Telephone: +86-25-84395164; Fax: +86-25-84395210

4 tables, 13 figures

Solid Phase Extraction

Immediately after incubation, the NOM samples were adjusted to pH < 2 by 99% H_2SO_4 before subjected to solid phase extraction (SPE). The SPE cartridge (Oasis HLB SPE cartridges, 12 cc, 1 g, Waters, Milford, MA) was conditioned with 5 mL methanol, two 5 mL aliquots of Milli-Q water sequentially, followed by sample loading. Approximately 500 mL sample was loaded onto each cartridge. For each treatment (1.0 L sample), 2 SPE cartridges were used. After finishing loading, each cartridge was rinsed with 2 mL Milli-Q water and blown to dryness under vacuum. The cartridge was then eluted with 5 mL methanol. The eluents were combined and 1 mL was splitted out for MS analysis. The rest was freeze dried using a Labconco FreeZone 2.5 (Kansas City, MO) and for FTIR analysis.

MS analysis

The enriched NOM nitration samples were first analyzed by an Agilent 1200 high performance liquid chromatography (HPLC) coupled with an Agilent 6410 triple quadrupole mass spectrometer (MS) with electrospray ionization (ESI) source (Agilent Technologies, USA). The sample was delivered to the ESI source without going through an HPLC column. The delivery solvent consisted of 50% methanol and 50% water, at a flow rate of 0.2 mL/min. The MS was operated at negative ionization and full scan (*m/z* 50-1000) mode. Detailed operational parameters were: capillary voltage of -3.5 kV, fragmentor 135 V, desolvation gas (nitrogen, ≥ 99.995%) flow rate 10 L/min, temperature 350 ℃, nebulizer (nitrogen, ≥ 99.995%) pressure 40 psi. Products ion scan (MS/MS) was performed for suspected nitrogenous products. The collision energy for MS/MS was optimized individually for each precursor ion.

The NOM nitration samples were also analyzed by a high-resolution hybrid quadrupole time-of-flight mass spectrometer (Triple TOF 5600+, AB Sciex, Foster City, CA), equipped with an ESI source. The sample was delivered to the ESI source without going through an HPLC column. MS was operated at the negative ion mode with mass range of *m/z* 100-1000. The ion source parameters were set as follows: nebulizer gas, 55 psi; heater gas, 55 psi; curtain gas, 35 psi; temperature, 550°C; ion spray voltage floating, -4500 V; declustering potential, -80 V; and collision energy, -10 V.

Quantification of nitrated byproducts from NOM

Nitrated byproducts in the samples were analyzed using an Agilent 1200 HPLC coupled with an Agilent 6410 triple quadrupole MS with ESI interface. The separation was accomplished on an Agilent Zorbax Eclipse Plus C18 column (150 mm × 2.1 mm i.d., 3.5 μm). The program of the delivery solvent consisting of methanol and pH 3 water (acidification by formic acid) was as following: methanol increasing linearly from 20% to 30% in the first 10 min; 30% to 70% in 10-20 min; 70% to 100% in 20-25 min; and maintaining 100% for 5 additional minutes. The flow rate was 0.2 mL/min. ESI was operated at negative mode with the parameters set as: capillary voltage of -3.5 kV, fragmentor 135 V, desolvation gas (nitrogen, ≥ 99.995%) flow rate 10 L/min, temperature 350 ℃, nebulizer (nitrogen, ≥ 99.995%) pressure 40 psi. MS was at multiple reaction monitoring (MRM) mode, the transition pair and collision energy for each analyte were experimentally determined and listed in Table S1.

Table S1. Parameter of MRM for suspected nitrogenous byproducts

Parent ion	Daughter ion	Collision energy
m/z 138	*m/z* 108	10
m/z 182	*m/z* 138	15
m/z 183	*m/z* 123	15
m/z 226	*m/z* 182	15

Model compounds analysis

Concentrations of 4-hydroxybenzoic acid (HBA) and nitrated byproducts were analyzed using a Hitachi L-2000 high performance liquid chromatography (HPLC, Hitachi, Japan) equipped with an Agilent Zorbax Eclipse Plus C18 column (250 mm * 4.6 mm i.d., 5 μm particle size) and an L-2455 diode array detector. Quantification was based on multipoint standard calibration curves. The isocratic eluent

was consisted of 50% H_2O and 50% methanol (both with 0.1% formic acid) at a flow rate of 1.0 mL/min (see Table S2). The concentrations of salicylic acid (SA) and nitrated byproducts were detected using the same HPLC instrument, while the gradient elution was consisted of 50% methanol and 50% water (both with 0.1% formic acid) in the first 4 minutes, then methanol increased from 50% to 60% in 4-4.1 min and maintained at 60% in 4.1-12 min, then it decreased to 50% in 12-12.1 min and kept at 50% at 12.1-15 min (see Table S3).

Table S2. HPLC analytical parameters for *p*-hydroxybenzoic acid (HBA) and nitrated byproducts.

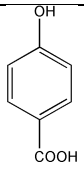
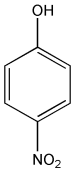
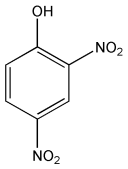
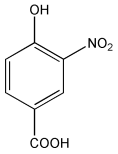
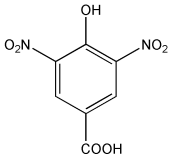
Compound	Molecular structure	Retention time (min)	Detection wavelength (nm)
p-hydroxybenzoic acid		3.75	252
4-nitrophenol		6.70	252
2,4-dinitrophenol		7.93	252
4-hydroxy-3-nitrobenzoic acid		6.34	236
4-hydroxy-3,5-dinitrobenzoic acid		3.65	252

Table S3. HPLC analytical parameters for Salicylic acid (SA) and nitrated byproducts.

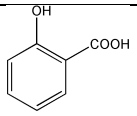
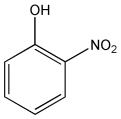
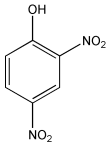
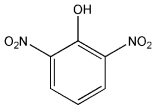
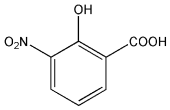
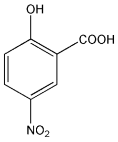
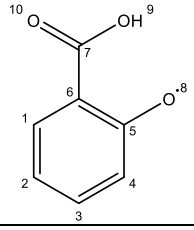
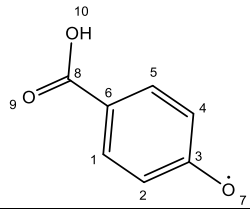
Compound	Molecular structure	Retention time (min)	Detection wavelength (nm)
Salicylic acid		8.71	231
2-nitrophenol		9.36	276
2,4-dinitrophenol		8.35	252
2,6-dinitrophenol		6.09	252
2-hydroxy-3-nitrobenzoic acid		4.02	252
2-hydroxy-5-nitrobenzoic acid		6.54	252

Table S4. Spin distribution of the radical intermediates obtained by DFT computation

			
Atom	Spin density (e/Å^3)	Atom	Spin density (e/Å^3)
1 C	-0.13851	1 C	-0.16532
2 C	0.406701	2 C	0.301123
3 C	-0.15805	3 C	-0.1164
4 C	0.303953	4 C	0.326046
5 C	-0.05861	5 C	-0.16912
6 C	0.282933	6 C	0.392106
7 O	0.377441	7 O	0.414308
8 C	-0.02999	8 H	0.006901
9 O	0.04481	9 H	-0.01597
10 O	-0.00126	10 H	-0.01747
11 H	0.005603	11 H	0.007075
12 H	-0.02191	12 C	-0.04054
13 H	0.00649	13 O	0.073622
14 H	-0.01645	14 O	0.003974
15 H	-0.00315	15 H	-0.00035

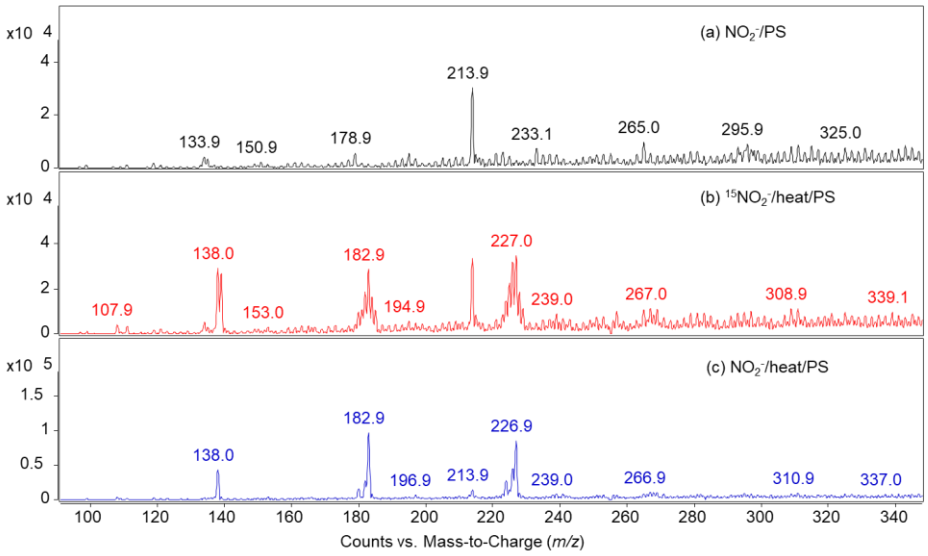


Figure S1. MS of NOM subjected to heat/PDS treatment in the presence of nitrite. NOM 4.96 mg/L as TOC, PDS 1.0 mM, NO_2^- 0.01 mM, pH 7, 24 h. (a) control, incubated at room temperature; (b) incubated at 60 ℃, 50% nitrite labeled with ^{15}N; (c) incubated at 60 ℃, nitrite unlabeled.

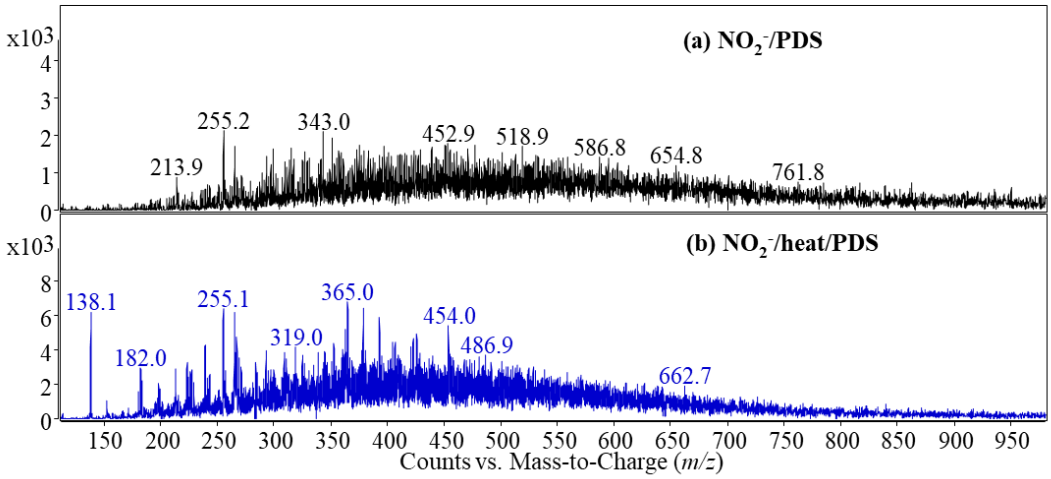


Figure S2. Mass spectra of NOM subjected to PDS treatment in the presence of nitrite. Experimental conditions: NOM 4.96 mg/L as TOC, PDS 1.0 mM, nitrite 0.5 mM. (a) Incubated at room temperature; (b) incubated at 60 ℃.

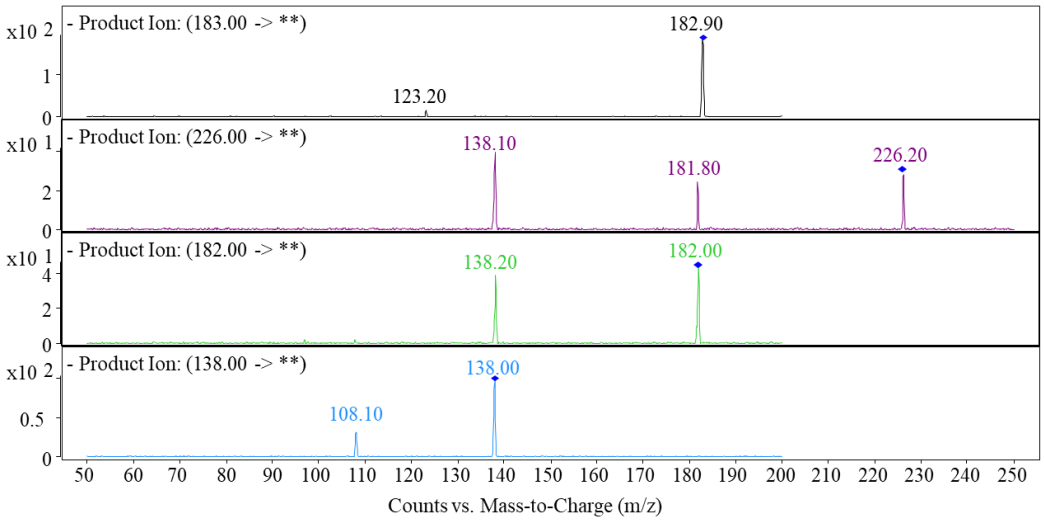


Figure S3. MS/MS of the nitroaromatic byproducts generated by heat/PDS treatment of NOM in the presence of nitrite.

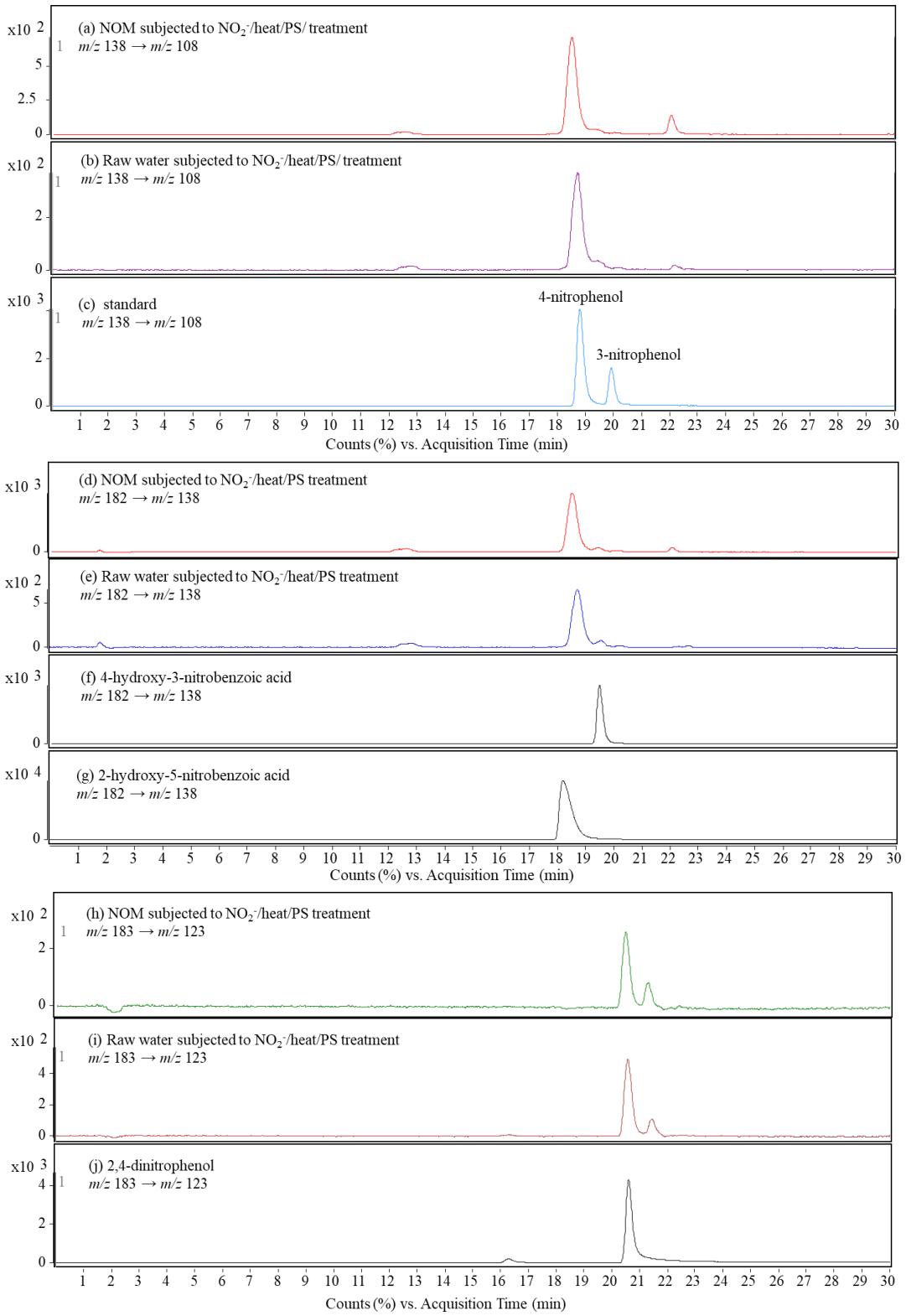


Figure S4. HPLC/MS/MS analysis of selected nitroaromatic byproducts and authentic standards.

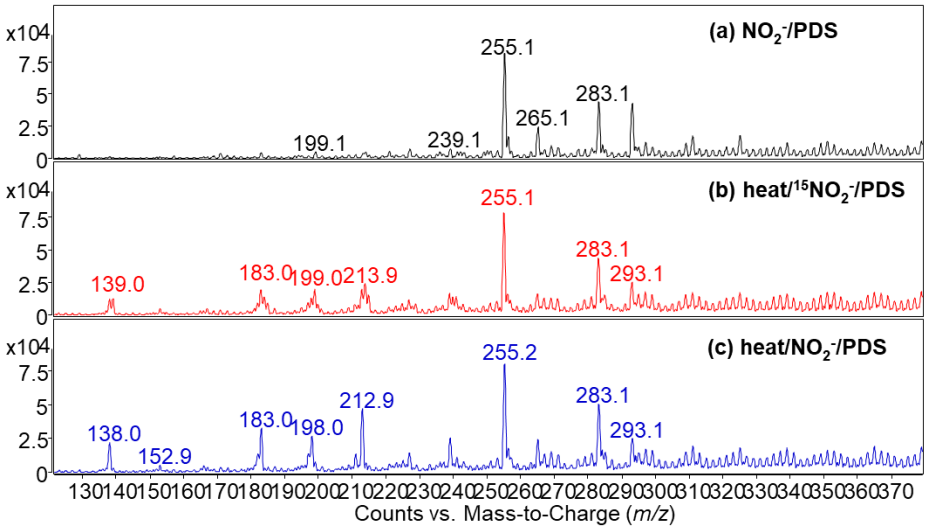


Figure S5. Mass spectra of the surface water subjected to heat/PDS treatment in the presence of nitrite, generated with negative ESI. The TOC and pH of the water was 9.74 mg/L and 8.2, respectively. Experimental conditions: PDS 1.0 mM, NO_2^- 0.5 mM, pH 7, 24 h. (a) control, incubated at room temperature; (b) incubated at 60 ℃, 50% nitrite labeled with ^{15}N; (c) incubated at 60 ℃.

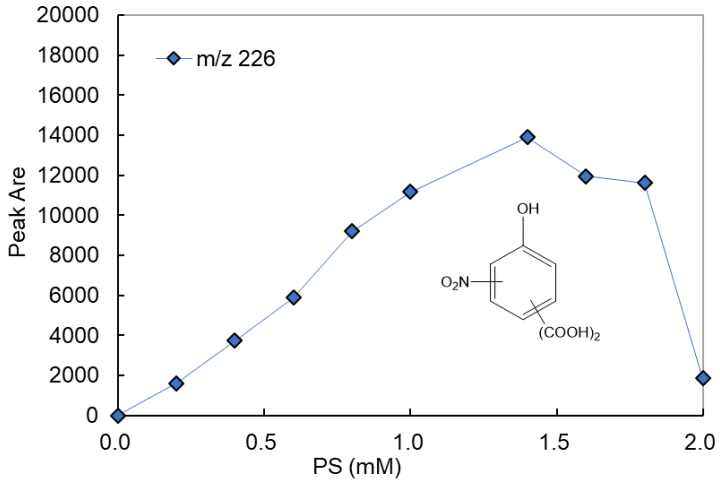


Figure S6. Formation of nitroaromatic byproducts (*m/z* 226) after NOM was treated in heat/PDS oxidation process in the presence of nitrite and varying PDS dose. Initial NOM 4.96 mg/L as TOC, NO_2^- 0.5 mM, pH 7, 60 ℃, 24 h incubation.

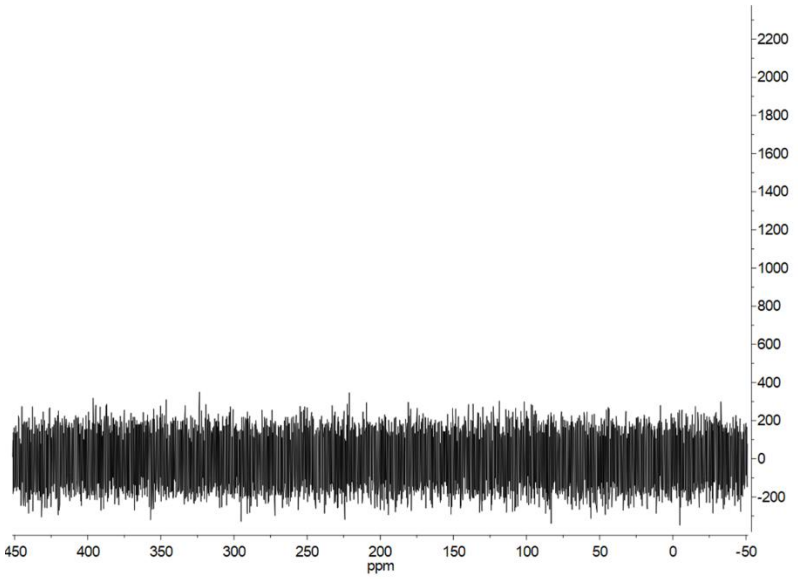


Figure S7. NMR of the NOM subjected to PDS treatment without heating in the presence of 100% ^{15}N labeled nitrite. Initial NOM 9.83 mg/L as TOC, PS 2.0 mM, NO_2^- 1 mM, pH 7, 24 h.

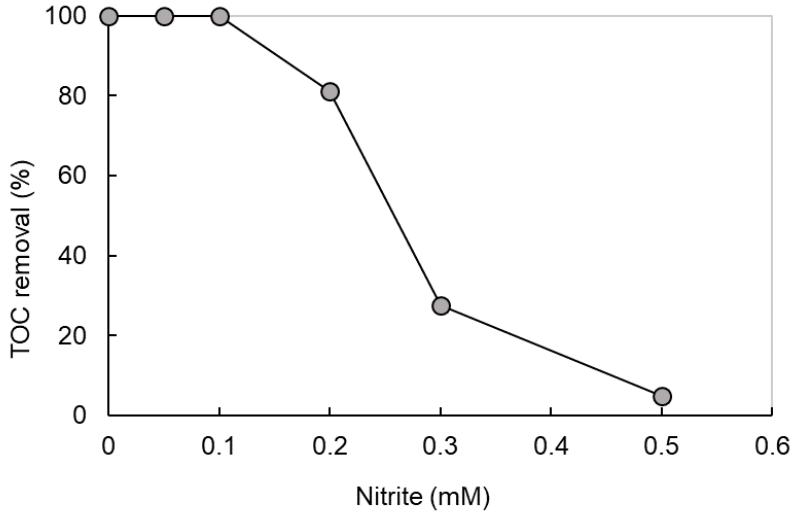


Figure S8. Removal of TOC after NOM was treated in heat/PDS in the presence of nitrite. Initial NOM 4.83 mg/L as TOC, PDS 1.0 mM, pH 7, 60 ℃, 24 h, NO_2^- varied from 0 to 0.5 mM.

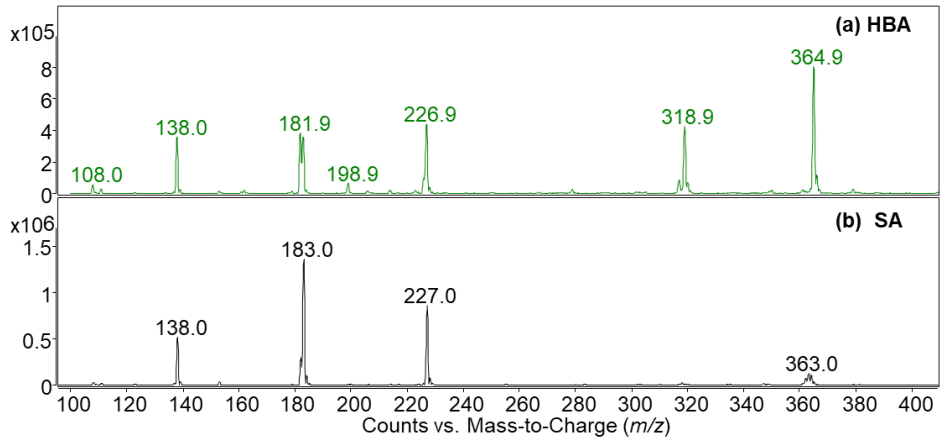


Figure S9. Mass spectra of (a) HBA and (b) SA subjected to heat/PDS treatment in the presence of nitrite, generated with negative ESI. Initial HBA or SA 50 μM, PDS 2.0 mM, NO_2^- 0.2 mM, pH 7, 60 ℃, 8 h.

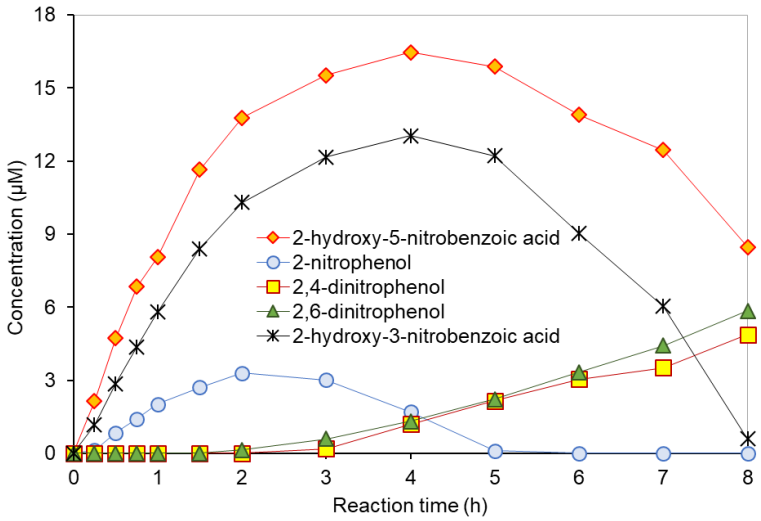


Figure S10. Time-course formation of nitrophenolic byproducts after SA was treated in heat/PDS oxidation process in the presence of nitrite. Initial SA 50 μM, PDS 2 mM, NO_2^- 0.2 mM, pH 7, 60 ℃.

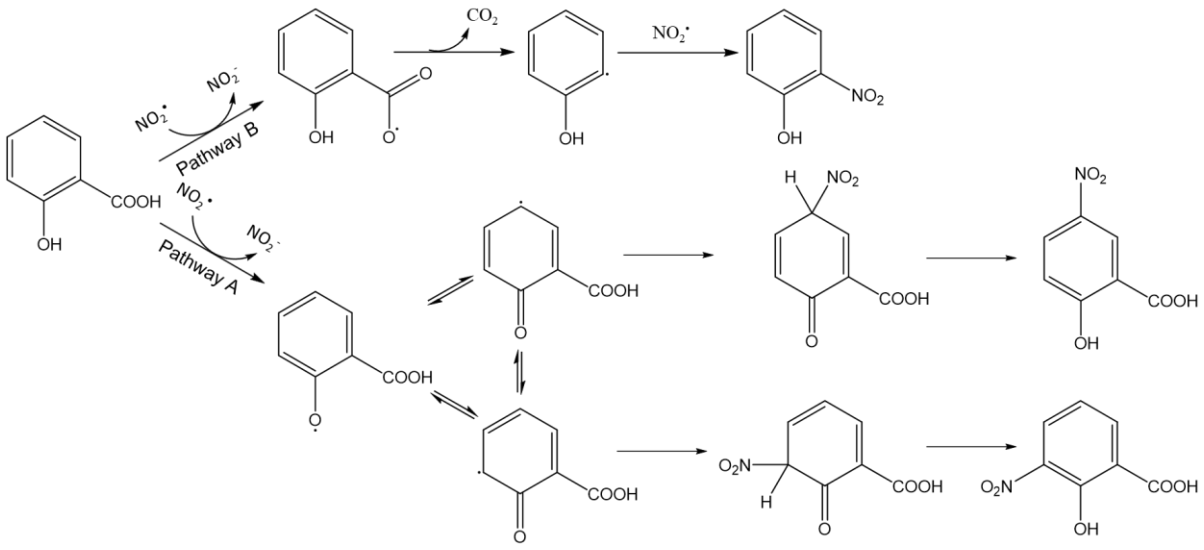


Figure S11. Nitration pathways of SA in heat/PDS oxidation process in the presence of nitrite.

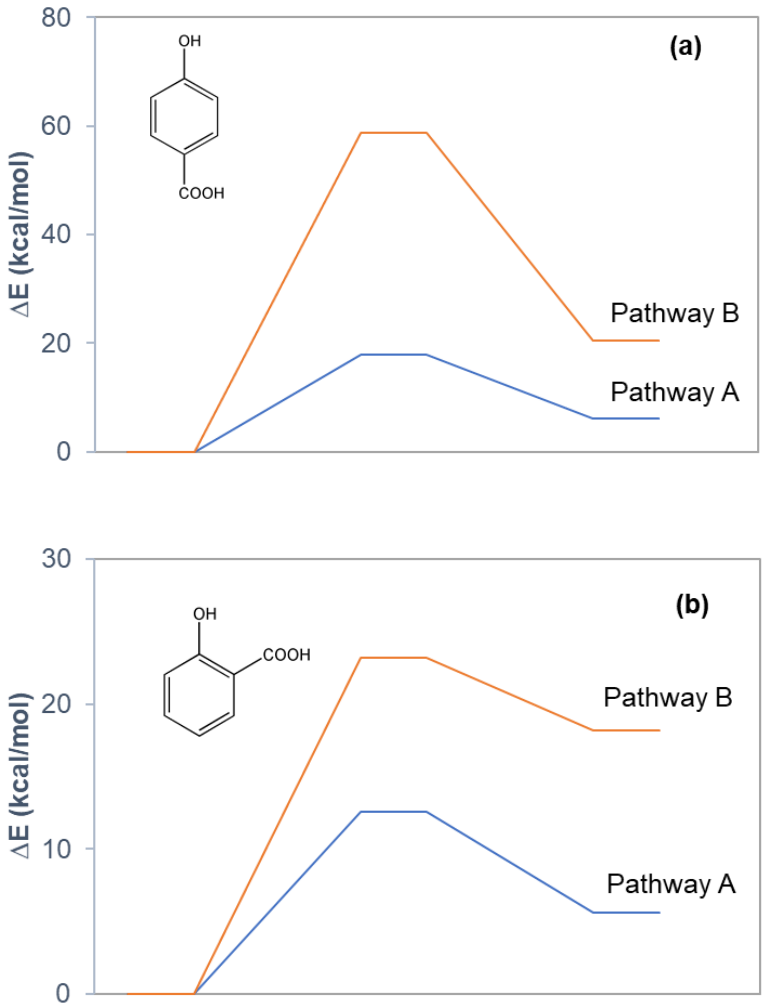


Figure S12. Computed free energy profiles for the H-abstraction pathways of (a) HBA and (b) SA upon the reaction with $NO_2^{\bullet}$.

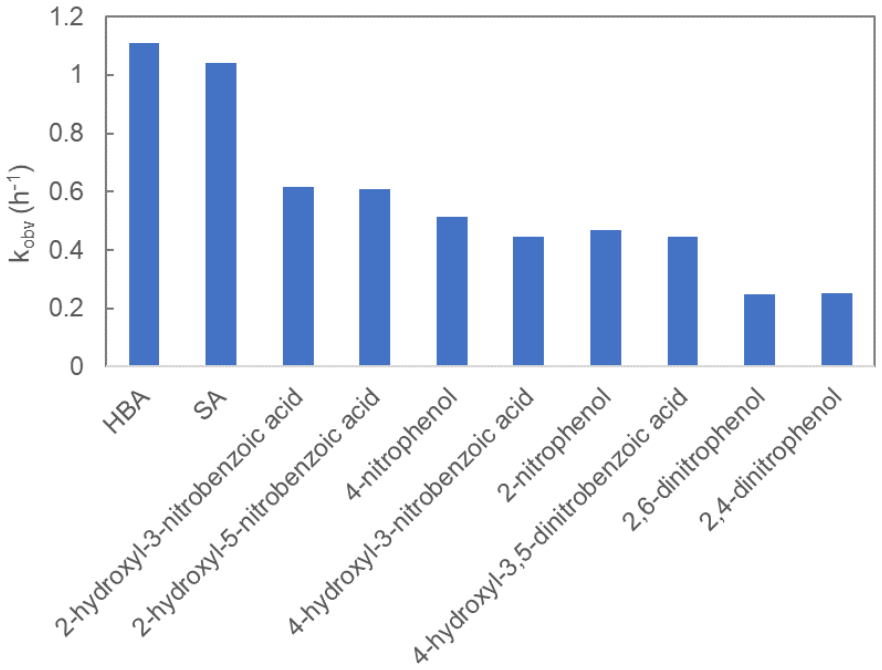


Figure S13. Pseudo first-order kinetic constants of the model compounds (HBA and SA) and their nitrated derivatives in heat/PDS oxidation process. Initial concentration 50 μM for each compound, PDS 2.0 mM, pH 7, 60 ℃.