



ADDITIONAL MATERIALS AVAILABLE ON THE HEI WEBSITE

Research Report 197

Cellular and Acellular Assays for Measuring Oxidative Stress Induced by Ambient and Laboratory-Generated Aerosols

Ng et al.

Additional Materials 3 Appendix C: Chamber Sample Analysis

Note: Additional Materials may appear in a different order than in the original Investigators' Report, and some remnants of their original names may be apparent. HEI has not changed the content of these documents, only their numeric identifiers. Additional Materials 3 was originally Appendix C.

These Additional Materials were not formatted or edited by HEI. This document was part of the HEI Review Committee's review process.

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Appendix C: Chamber sample analysis

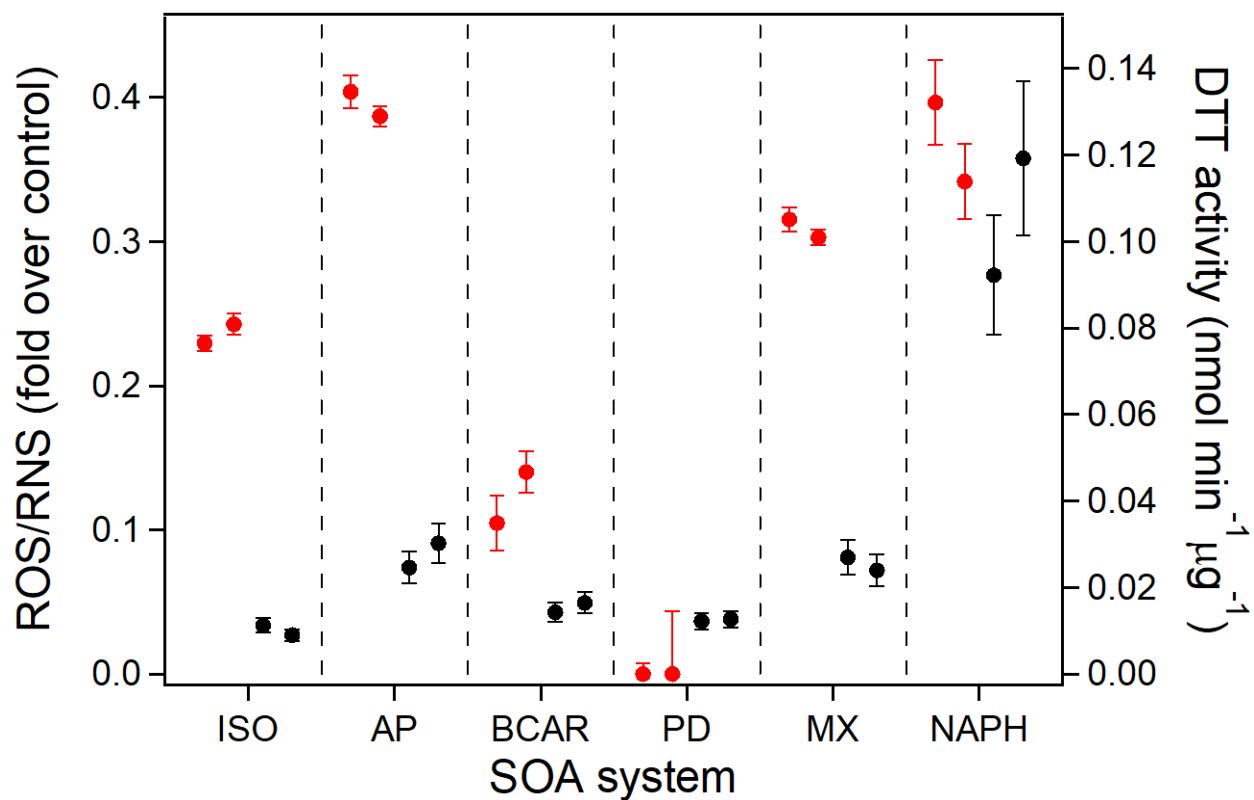


Figure C1. ROS/RNS produced as a result of exposure and oxidative potential as measured by dithiothreitol (DTT activity) for chamber experiments repeated to ensure reproducibility. SOA were generated from various precursors: isoprene (ISO), α -pinene (AP), β -caryophyllene (BCAR), pentadecane (PD), *m*-xylene (MX), and naphthalene (NAPH) under dry, $\text{RO}_2 + \text{HO}_2$ dominant formation conditions.

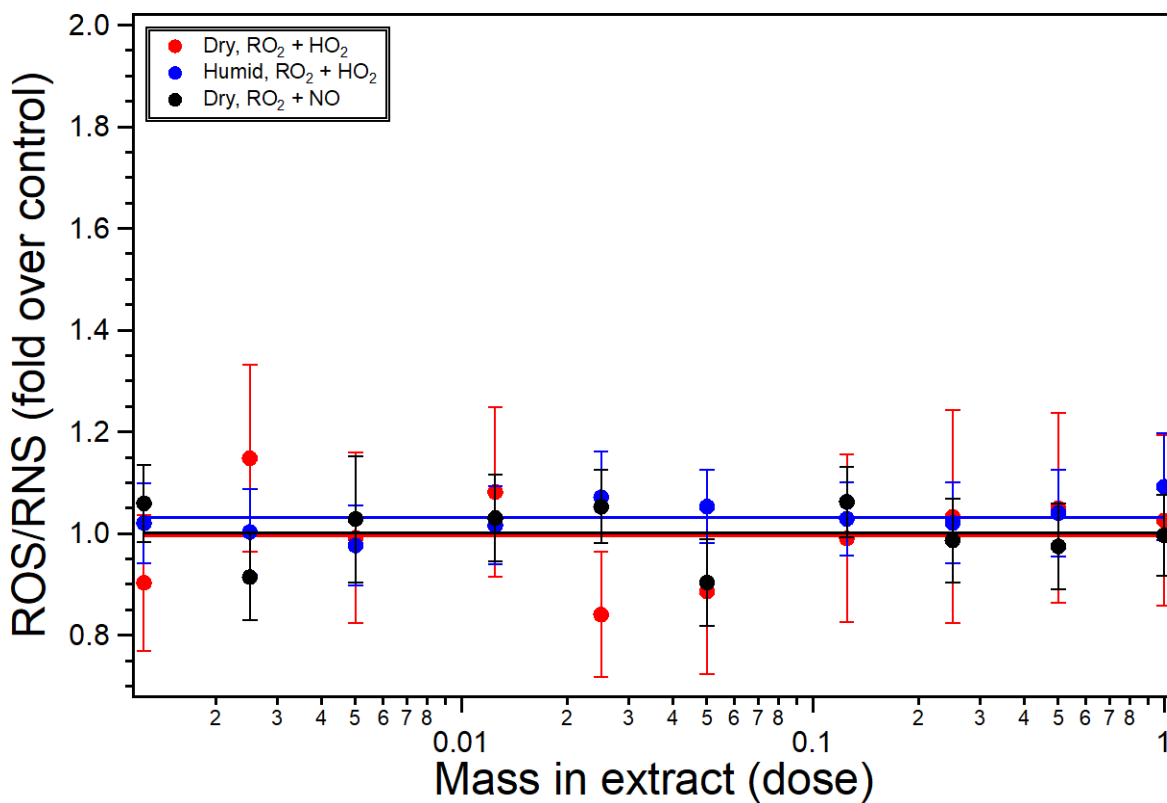


Figure C2. ROS/RNS produced as a result of exposure to background filters containing only seed particle and OH precursor (H₂O₂ or HONO) at experimental concentrations. ROS/RNS is expressed as a fold increase over probe-treated control cells exposed to stimulant-free media.

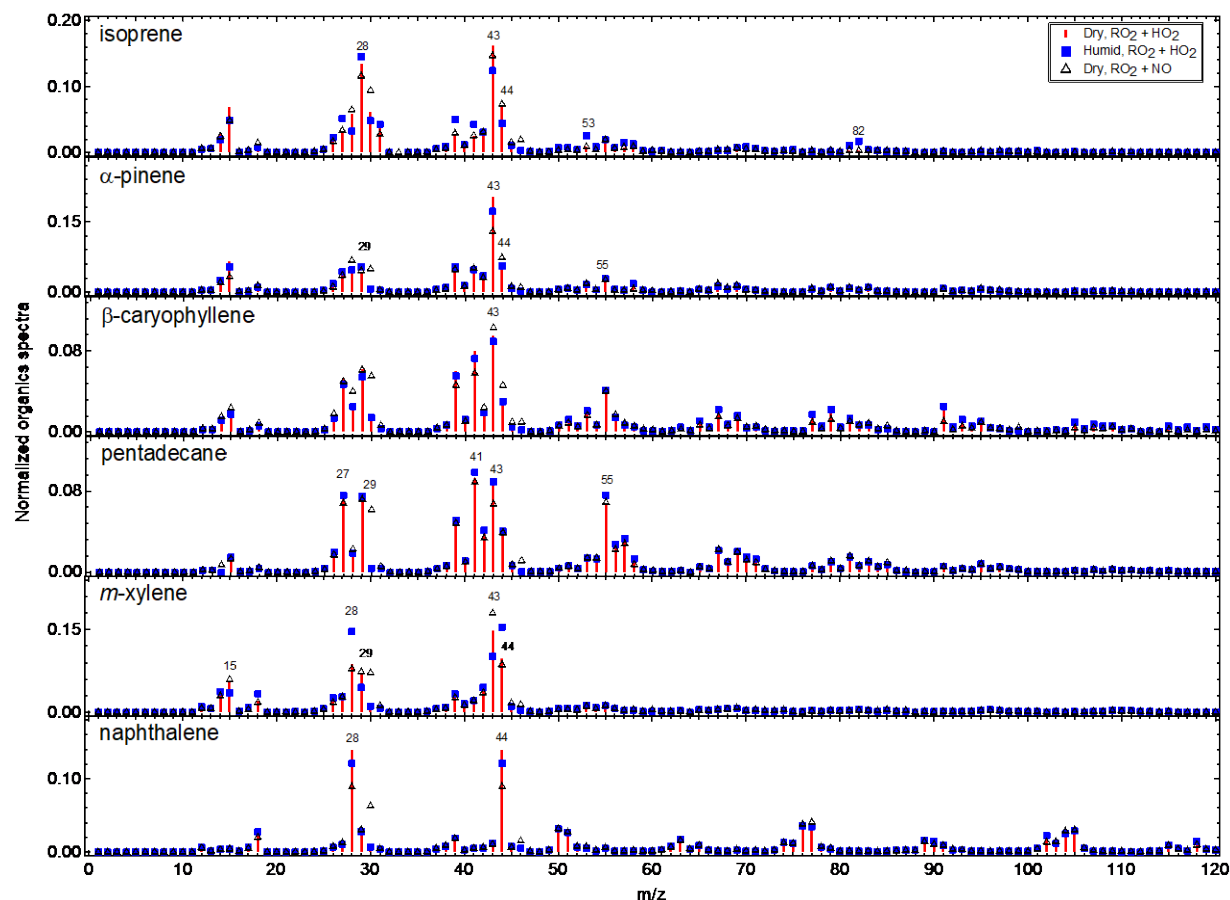


Figure C3. Aerosol mass spectra for SOA formed from the photooxidation of various precursors (ISO: isoprene, AP: α -pinene, BCAR: β -caryophyllene, PD: pentadecane, MX: *m*-xylene, and NAPH: naphthalene) under different formation conditions (**red bars**: dry, $\text{RO}_2 + \text{HO}_2$; **blue squares**: humid, $\text{RO}_2 + \text{HO}_2$; and **black triangles**: dry, $\text{RO}_2 + \text{NO}$). Characteristic fragments for each system are labeled.

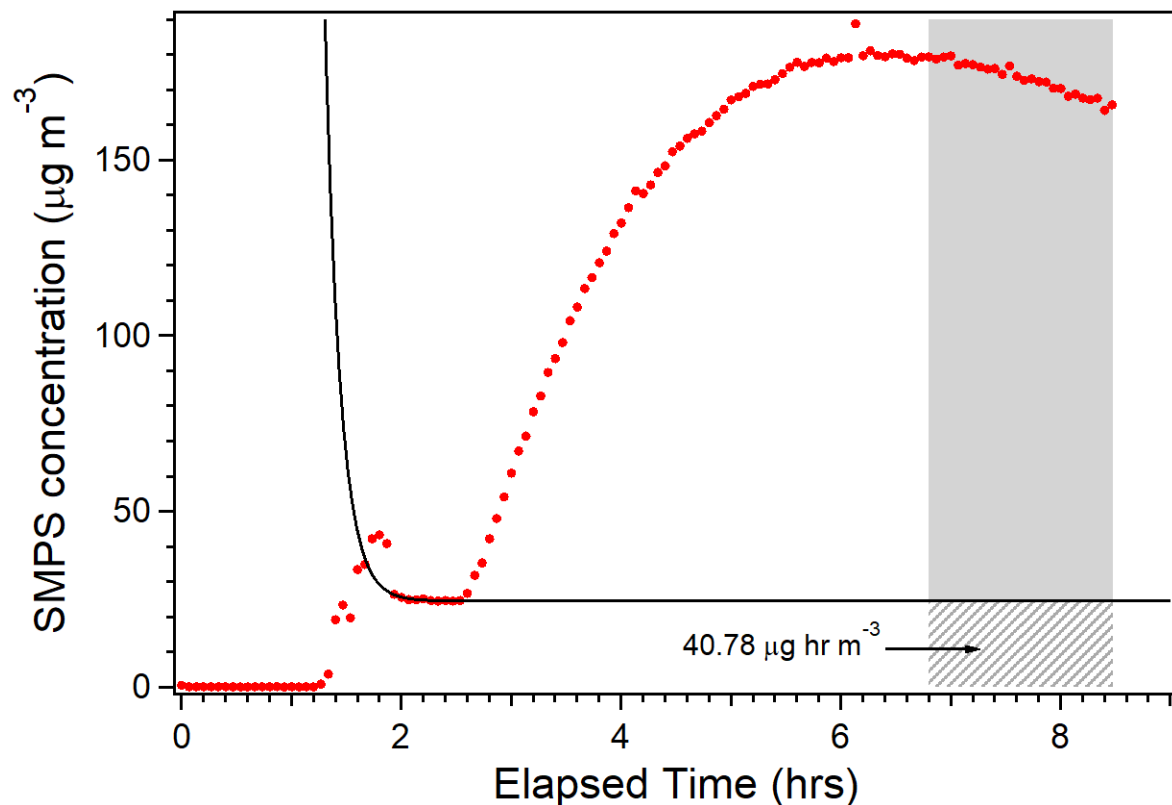


Figure C4. Method for estimating the mass of seed collected onto filters for naphthalene SOA formed in the presence of iron sulfate seed. A double exponential (black line) was used to fit seed concentrations obtained from the SMPS as a function of time (red markers). The fitted seed concentration as a function of time (black line) was then integrated over the filter collection period (shaded region). To obtain the total mass of seed collected, the integral ($40.78 \mu\text{g m}^{-3}$) was multiplied by the volumetric flow rate ($1.72 \text{ m}^3 \text{ hr}^{-1}$, for an estimated total seed mass of $70.14 \mu\text{g}$ on the filter for this experiment). Source: Tuet et al. 2017; licensed under CC BY 4.0.

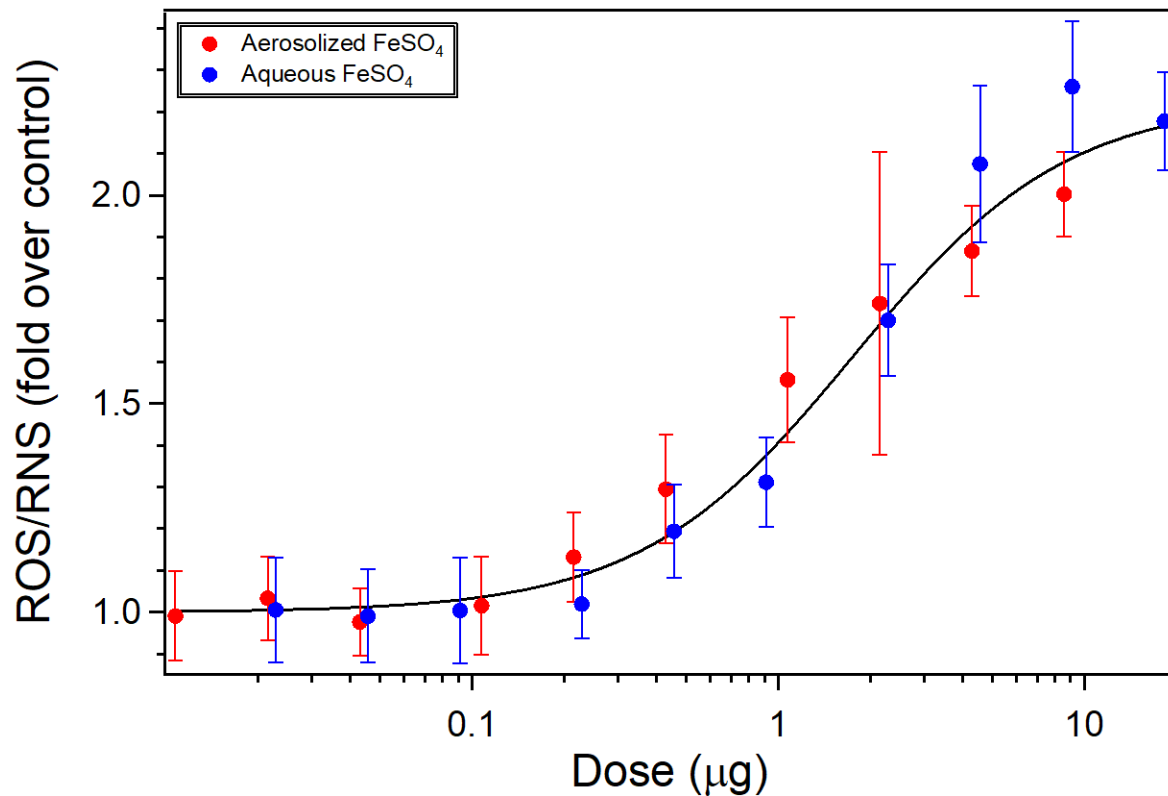


Figure C5. ROS/RNS levels for iron sulfate (**red**: aerosolized into the chamber at experimental concentration, collected onto a filter, and extracted into media; **blue**: aqueous seed solution diluted in media). ROS/RNS is expressed as a fold increase over probe-treated control cells incubated with stimulant-free media. Source: Tuet et al. 2017; licensed under CC BY 4.0.

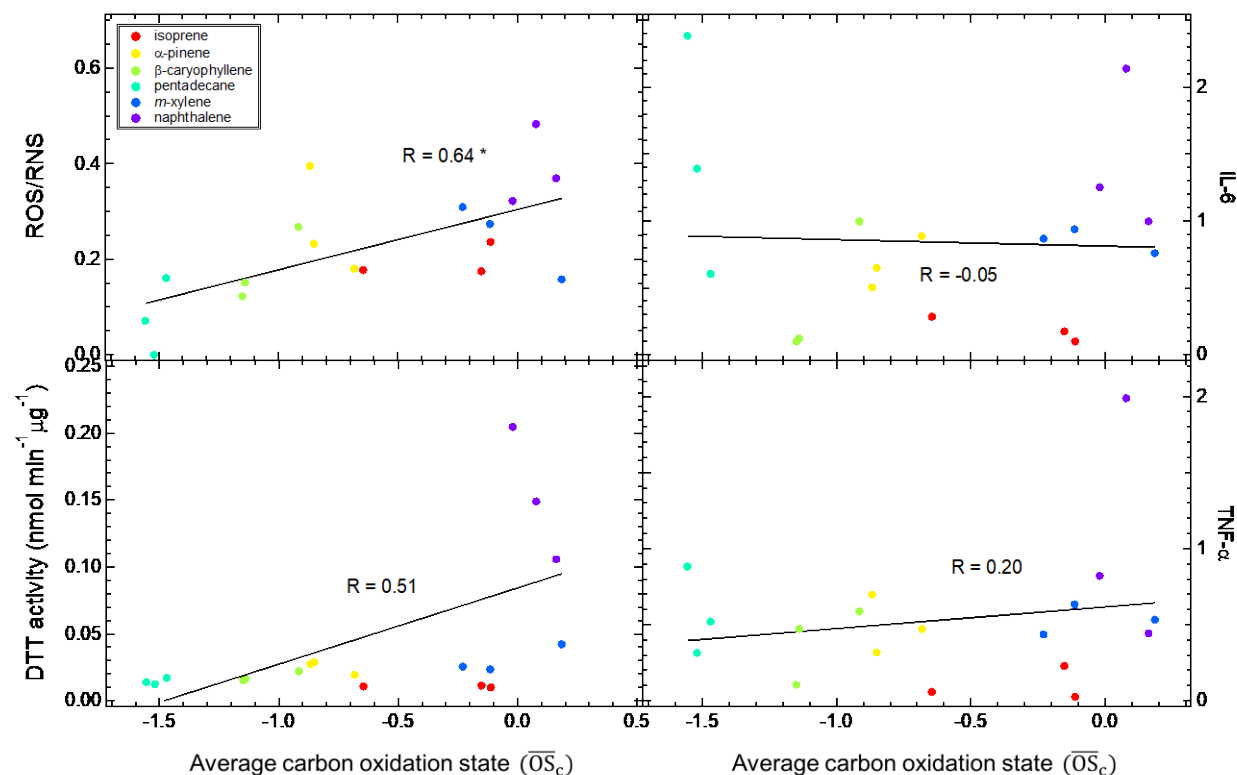


Figure C6. Correlations between aerosol average carbon oxidation state (\overline{OS}_c) and oxidative properties, including DTT activity, ROS/RNS production, IL-6 production, and TNF- α production. DTT activities are given per mass of aerosol, while cellular inflammatory responses are given as the area under the dose-response curve (AUC). SOA were generated from various precursors (isoprene, α -pinene, β -caryophyllene, pentadecane, *m*-xylene, and naphthalene) under different formation conditions (dry, $RO_2 + HO_2$; humid, $RO_2 + HO_2$; dry, $RO_2 + NO$). Linear fits and corresponding Pearson's correlation coefficient are given. * indicates significance, $p < 0.05$.

Table C1. Blank corrected dithiothreitol decay (nmol min⁻¹) for chamber background filters.

Formation condition	Precursor	DTT decay (nmol min ⁻¹)
Dry, RO ₂ + HO ₂	isoprene	-0.0027
	α -pinene	-0.014
	β -caryophyllene	-0.0086
	pentadecane	-0.00042
	<i>m</i> -xylene	-0.0044
	napthalene	-0.042
Humid, RO ₂ + HO ₂	isoprene	-0.0010
	α -pinene	-0.0054
	β -caryophyllene	-0.0036
	pentadecane	-0.0038
	<i>m</i> -xylene	-0.0066
	napthalene	0.014
Dry, RO ₂ + NO	isoprene	0.0069
	α -pinene	-0.0049
	β -caryophyllene	-0.0025
	pentadecane	0.0026
	<i>m</i> -xylene	0.0091
	napthalene	0.011

Table C2. Sampling flow rate and volume of air collected for chamber filters.

Experiment	SOA precursor	Seed particle ^{a,b,c}	OH precursor	Relative humidity (%)	Q (m ³ hr ⁻¹)	V (m ³)
1	isoprene	AS	H ₂ O ₂	<5%	1.71	2.68
2	α -pinene	AS	H ₂ O ₂	<5%	1.72	2.10
3	β -caryophyllene	AS	H ₂ O ₂	<5%	1.72	2.01
4	pentadecane	AS	H ₂ O ₂	<5%	1.73	2.74
5	<i>m</i> -xylene	AS	H ₂ O ₂	<5%	1.74	2.73
6	naphthalene	AS	H ₂ O ₂	<5%	1.74	2.09
7	isoprene	MS + SA	H ₂ O ₂	<5% ^a	1.74	2.83
8	α -pinene	AS	H ₂ O ₂	40%	1.73	3.86
9	β -caryophyllene	AS	H ₂ O ₂	42%	1.33	2.39
10	pentadecane	AS	H ₂ O ₂	45%	1.72	2.90
11	<i>m</i> -xylene	AS	H ₂ O ₂	45%	1.72	3.04
12	naphthalene	AS	H ₂ O ₂	44%	1.74	3.30
13	isoprene	AS	HONO	<5%	1.71	3.25
14	α -pinene	AS	HONO	<5%	1.71	2.85
15	β -caryophyllene	AS	HONO	<5%	1.70	2.90
16	pentadecane	AS	HONO	<5%	1.75	2.83
17	<i>m</i> -xylene	AS	HONO	<5%	1.72	2.83
18	naphthalene	AS	HONO	<5%	1.72	2.83
19	naphthalene	AS	H ₂ O ₂	51%	1.73	2.83
20	naphthalene	AS	H ₂ O ₂	49%	1.75	2.83
21	naphthalene	AS	H ₂ O ₂	54%	1.75	2.63
22	naphthalene	AS	H ₂ O ₂	53%	1.73	2.83
23	naphthalene	FS	H ₂ O ₂	50%	1.73	3.11
24	naphthalene	FS	H ₂ O ₂	48%	1.75	2.83
25	naphthalene	FS	H ₂ O ₂	52%	1.72	2.83
26	naphthalene	FS	H ₂ O ₂	51%	1.73	2.83

^a AS: Ammonium sulfate seed (15 mM (NH₄)₂SO₄); ^b MS + SA: Acidic seed (8 mM MgSO₄ and 16 mM H₂SO₄) was used instead of 8 mM (NH₄)₂SO₄; ^c FS: Iron sulfate seed (15 mM FeSO₄)