Supporting Information for

Intracellular Organic Matter from Cyanobacteria as a Precursor for Carbonaceous and Nitrogenous Disinfection Byproducts

Eric C. Wert [1,2*], and Fernando L. Rosario-Ortiz [2]

[1] Southern Nevada Water Authority (SNWA), P.O. Box 99954, Las Vegas, NV 89193-9954 USA

[2] Department of Civil, Environmental and Architectural Engineering, University of Colorado, Boulder, Boulder, CO 80309

* Author to whom correspondence should be addressed

Tel.: + 1-702-856-3669

Email: eric.wert@snwa.com

This Supporting Information contains 2 texts, 5 tables, and 6 figures for further information regarding the analytical methods (Text S1, Table S1) experimental conditions of Cl2 and NH2Cl testing (Tables S2-S3), testing with cyanobacteria cells (Text S2, Tables S4-S5) and the results from FP testing (Figures S3-S6). This information is available free of charge via the Internet at http://pubs.acs.org.

Text S1. Description of Algae Culturing Method

Culturing of cyanobacteria was performed inside a growth chamber (Geneva Scientific, Fontana, WI, USA) with constant light (12 hrs light followed by 12 hrs of darkness) and temperature (22°C) conditions. The cyanobacteria were cultured in either BG-11 growth media (for OSC and LYN) or Bold 3N growth media (for MA). A digital flow cytometer (FlowCAM®, Fluid Imaging Technologies, Yarmouth, ME, USA) was used to quantify the cell concentration in the stock cell suspension and monitor the growth cycle.

Text S2. DBP testing following ozone oxidation of cyanobacteria cells added to CRW.

Additional testing was conducted to evaluate the DBP formation from IOM released during cell damage from ozone oxidation. Cyanobacteria cells (MA, OSC, or LYN) were added to CRW followed by ozonation (0-5 mg/L) to release IOM through cell damage. Upon completion of first order ozone decay (<1 hr), the samples were filtered using a 0.7 µm GFF. The filtrate was used to conduct DBP testing using either 3 mg/L of NH2Cl or Cl2. After a 24 hour reaction period, the samples were quenched, preserved, and analyzed for DBPs, DOC, and DN.

The results showed that very little DOC was released (<0.3 mg/L) from the cyanobacteria cells under the cell concentrations and ozone oxidation conditions evaluated. As a result of the low DOC release and low NH2Cl exposure, nitrosamine concentrations were below the MRL (Table S3). Other DBPs resulting from chlorination (TOX, THMs, HAA5, TCNM) decreased when using a low ozone dose (0.63-1.25 mg/L), likely due to oxidation of precursor material found in the background NOM present in

CRW. At greater ozone dosages (2.5-5.0 mg/L), DBP formation began to increase. The increase can likely be attributed to greater cell damage and release of IOM, although bulk DOC measurements did not appear to provide statistically significant differences in DOC.

Table S1. – Overview of Analytical Methods.

Water Quality Parameter	Sample Bottle	Quenching/ Preservation	Analysis Method	Method Description	MRL
Dissolved Organic Carbon	40 mL Amber Glass Vial	160 µL HCl	SM 5310B	Combustion-infrared	<0.2 mg/L
Dissolved Nitrogen					<0.2 mg/L
UV Absorbance at 254 nm	40 mL Amber Glass Vial	None	SM 5910	Absorbance	<0.002/cm
Assimilable Organic Carbon	1L Amber Glass Bottle	None	Weinrich et al. (2009) AEM, 75(23), 7385-7390.	Bioluminescence	< 1 µg/L
Total Organic Halides	500 mL Amber Glass Bottle	80 mg/L Sodium Thiosulfate	SM 5320B	Activated carbon adsorption, Microcoulometric-titration detection	<20 µg/L
Trihalomethanes					
Chloroform	(2) 40 mL clear glass vials	30 µL of 10% sodium thiosulfate	USEPA Method 524.2	Gas Chromatography with Mass Spectrometry (GC/MS)	<0.5 µg/L
Bromodichloromethane					<0.5 µg/L
Dibromochloromethane					<0.5 µg/L
Bromoform					<0.5 µg/L

Haloacetic Acids					
Chloroacetic acid	(2) 60 mL Amber Glass Vials	6 mg NH4Cl	USEPA Method 552.2	Gas Chromatography with Electron Capture Detection (GC/ECD)	<2.0 µg/L
Dichloroacetic acid					<1.0 µg/L
Trichloroacetic acid					<1.0 µg/L
Bromoacetic acid					<1.0 µg/L
Dibromoacetic acid					<1.0 µg/L
Bromochloroacetic acid					<1.0 µg/L
Bromodichloroacetic acid					<1.0 µg/L
Chlorodibromoacetic acid					<1.0 µg/L
Tribromoacetic acid					<1.0 µg/L
Haloacetonitriles					
Bromochloroacetonitrile	(2) 60 mL Amber Glass Vials	6 mg NH4Cl	USEPA Method 551.1	Liquid-liquid extraction, GC/ECD	<1 µg/L
Dibromoacetonitrile					<1 µg/L
Dichloroacetonitrile					<1 µg/L
Trichloroacetonitrile					<1 µg/L
Trichloronitromethane					<1 µg/L
Nitrosamines					
N-nitrosodimethylamine	1L Amber Glass Bottle	80 mg Sodium Thiosulfate, 1g Sodium Azide	Holady, et al., (2012) *Am. Lab*. 44(3), 25-30.	GC-MS/MS	<2.5 ng/L
N-nitrosomethylethylamine					<2.5 ng/L
N-nitrosodiethylamine					<5 ng/L
N-nitrosodipropylamine					<10 ng/L
N-nitrosomorpholine					<5 ng/L
N-nitrosopyrrolidine					<10 ng/L
N-nitrosopiperidine					<5 ng/L
N-nitrosodibutylamine					<10 ng/L
N-nitrosodiphenylamine					<10 ng/L

Aldehydes					
Acetaldehyde					<1 µg/L
Butanal					<1 µg/L
Formaldehyde	(3) 40 mL Amber Glass Vials	25 mg NH_4Cl 22 mg $CuSO_4$	USEPA Method 556	GC/ECD	<5 µg/L
Glyoxal					<10 µg/L
M-Glyoxal					<10 µg/L
Pentanal					<1 µg/L
Propanal					<1 µg/L

Table S2. Summary of FP Testing Conditions with Free Chlorine and Measured FCl_2 Residuals after 7 days.

Water Source	IOM Addition (mg/L)	O_3 Dose (mg/L)	FCl_2 Dose (mg/L)	Bromide Addition (µg/L)	FCl_2 Residual @ 7 days (mg/L)		
					MA	OSC	LYN
Buffered Milli-Q	0	-	5	-	5.0	4.8	4.1
	1.0	-	15	-	11.1	8.5	7.9
	2.0	-	25	-	7.2	12.0	10.8
	3.0	-	35	-	7.6	-	13.4
	0	-	5	100	5.1	4.7	4.2
	1.0	-	15	100	11.1	8.5	7.7
	2.0	-	25	100	6.6	11.8	11.0
	3.0	-	35	100	7.0	-	-
	1.0	0.6	21	100	10.8	-	13.4
CRW (~2.5 mg/L DOC)	0	-	6	Ambient	3.2	2.1	2.0
	0	1.5	6	Ambient	3.0	2.1	2.1
	1.0	-	21	Ambient	8.0	9.0	9.4
	1.0	2.1	21	Ambient	8.2	9.8	9.9
	1.0	2.1	21	Ambient	8.4	10.0	9.9

Table S3. Summary of FP Testing Conditions with Chloramines and Measured NH_2Cl Residuals after 7 days.

Water Source	IOM Addition (mg/L)	O_3 Dose (mg/L)	NH_2Cl Dose (mg/L)	Bromide Spike (µg/L)	NH_2Cl Residual @ 7 days (mg/L)		
					MA	OSC	LYN
Buffered Milli-Q	0	-	5	-	3.90	3.55	3.95
	1	-	12	-	7.75	7.90	8.20
	2	-	19	-	13.10	11.40	11.80
	3	-	26	-	17.10	-	15.40
	0	-	5	100	3.95	3.60	3.80
	1	-	12	100	7.80	7.80	8.10
	2	-	19	100	13.00	13.40	11.70
	3	-	26	100	17.00	-	
	1.0	0.6	16	100	11.10	-	10.80
CRW (~2.5 mg/L DOC)	0	-	4	Ambient	3.24	3.25	3.35
	0	1.5	4	Ambient	3.02	2.90	3.05
	1.0	-	16	Ambient	13.00	11.90	12.90
	1.0	2.1	16	Ambient	13.00	11.30	12.30
	1.0	2.1	16	Ambient	13.00	11.50	12.50

Table S4. Nitrosamine formation following O3/NH2Cl treatment of cyanobacteria cells added to CRW.

Cyanobacteria	Ozone	O3:DOC	DOC	DN	Nitrosamines (24 hrs)								
(Cells/mL)	Dose				NDMA	NMEA	NDEA	NDPA	NMOR	NPYR	NPIP	NDBA	NDPhA
	(mg/L)	Ratio	(mg/L)	(mg/L)	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
MA	0	0	2.66	0.53	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
(200,000/mL)	0.63	0.25	2.99	0.62	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
	1.25	0.5	2.85	0.54	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
	1.88	0.75	2.78	0.56	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	5.0	< 10	< 10
	2.5	1	2.84	0.56	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
	5.0	2	2.85	0.61	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
OSC	0	0	2.38	1.87	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
(1,400/mL)	0.63	0.25	2.37	1.82	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
	1.25	0.5	2.44	1.84	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
	1.88	0.75	2.33	1.80	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
	2.5	1	2.35	1.85	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
	2.5*	1	2.29	1.77	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
LYN	0	0	2.60	2.00	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
(2,800 /mL)	0*	0	2.52	1.97	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
	0.63	0.5	2.55	1.94	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
	1.25	0.75	2.48	1.93	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
	2.5	1	2.49	1.95	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10
	2.5*	1	2.32	1.90	< 2.5	< 2.5	< 5.0	< 10	< 5.0	< 10	< 5.0	< 10	< 10

*No cyanobacteria cells added.

Table S5. DBP formation following O3/Cl2 treatment of cyanobacteria cells added to CRW.

Isolate (Cells/mL)	Ozone Dose (mg/L)	O3:DOC Ratio	TOC (mg/L)	TN (mg/L)	TOX (µg Cl/L)	THM4 (µg/L)	HAA5 (µg/L)	TCNM (µg/L)
MA	0	0	2.64	0.49	180	81	25	1.56
(200,000/mL)	0.63	0.25	2.95	0.55	180	83	26	3.55
	1.25	0.5	2.80	0.54	170	73	20	1.66
	1.88	0.75	2.79	0.53	160	72	21	1.45
	2.5	1	2.89	0.54	180	75	24	2.16
	5.0	2	2.87	0.60	210	76	37	5.16
OSC	0	0	2.48	0.59	140	79	26	2.30
(1,400/mL)	0.63	0.25	2.48	0.59	110	62	18	1.58
	1.25	0.5	2.44	0.57	100	59	17	1.18
	1.88	0.75	2.40	0.59	120	60	18	1.31
	2.5	1	2.38	0.58	120	65	30	2.89
	2.5*	1	2.17	0.54	87	50	15	0.90
LYN	0	0	2.58	0.59	170	75	25	1.77
2,800/mL	0*	0	2.53	0.57	180	76	23	1.73
	0.63	0.5	2.45	0.58	150	62	18	1.02
	1.25	0.75	2.48	0.58	160	62	19	1.47
	2.5	1	2.45	0.57	140	62	20	1.41
	2.5*	1	2.34	0.55	130	58	18	0.71

*No Cells Added

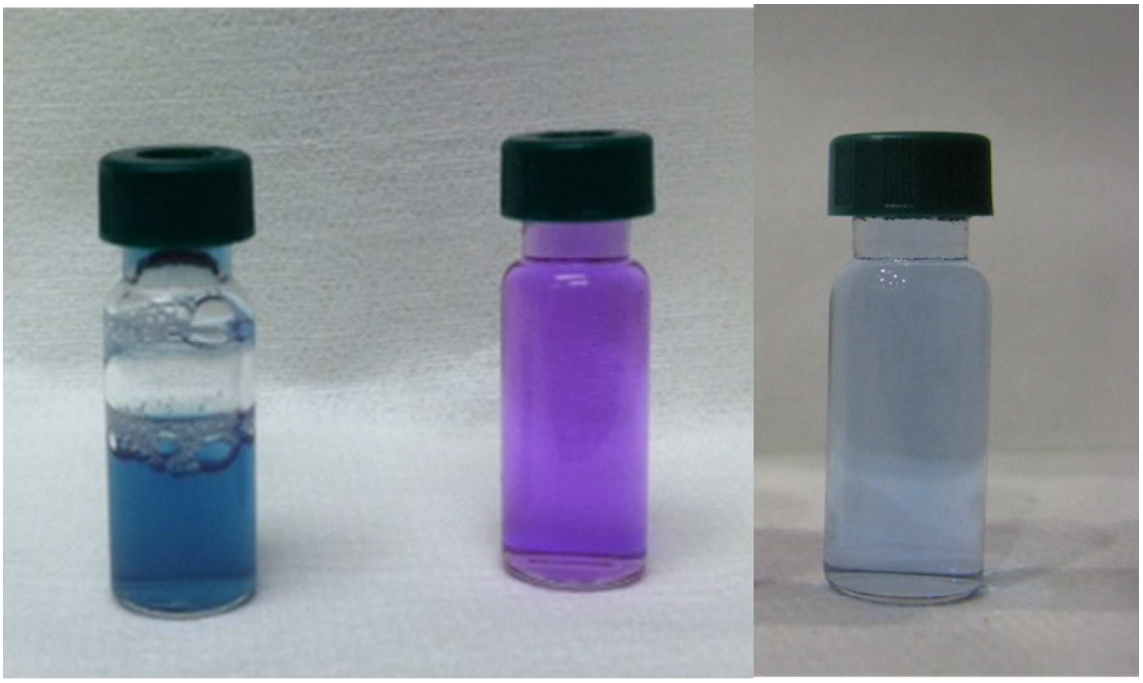

Figure S1. Photo of IOM standards: *Microcystis aeruginosa* (left, 320 mg/L), *Oscillatoria sp*. (center, 200 mg/L), and *Lyngbya sp*. (right, 115 mg/L).

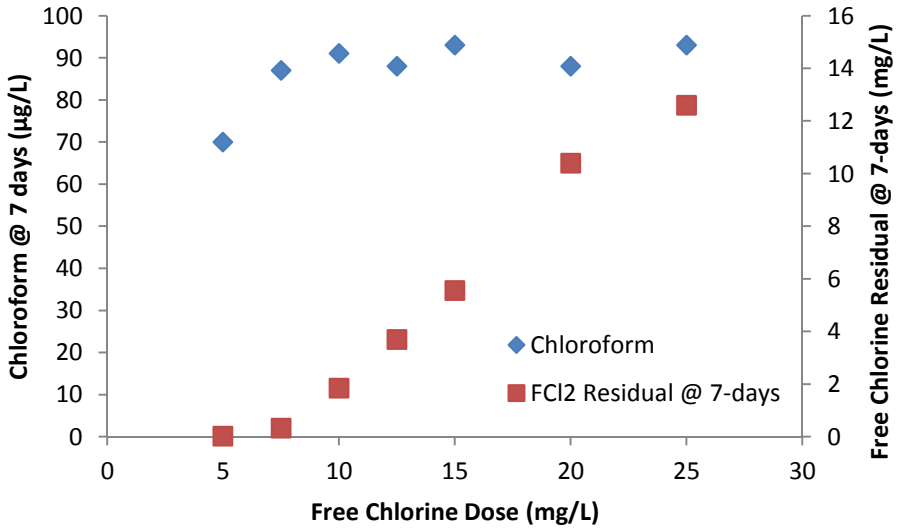

Figure S2. Effect of terminal free chlorine residual on overall chloroform yield after 7-days of exposure.

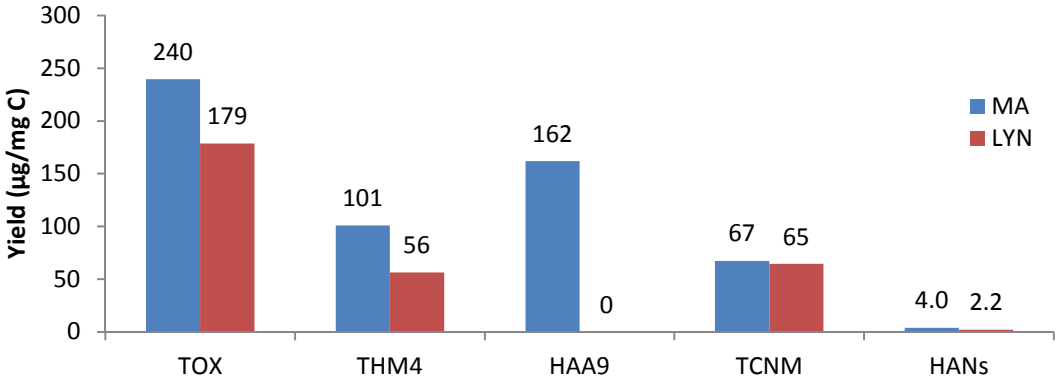

Figure S3. DBP yields after O_3/FCl_2 treatment in MQ water (pH=7.5, T=22-24°C, bromide=100 µg/L) with the addition of IOM from MA and LYN.

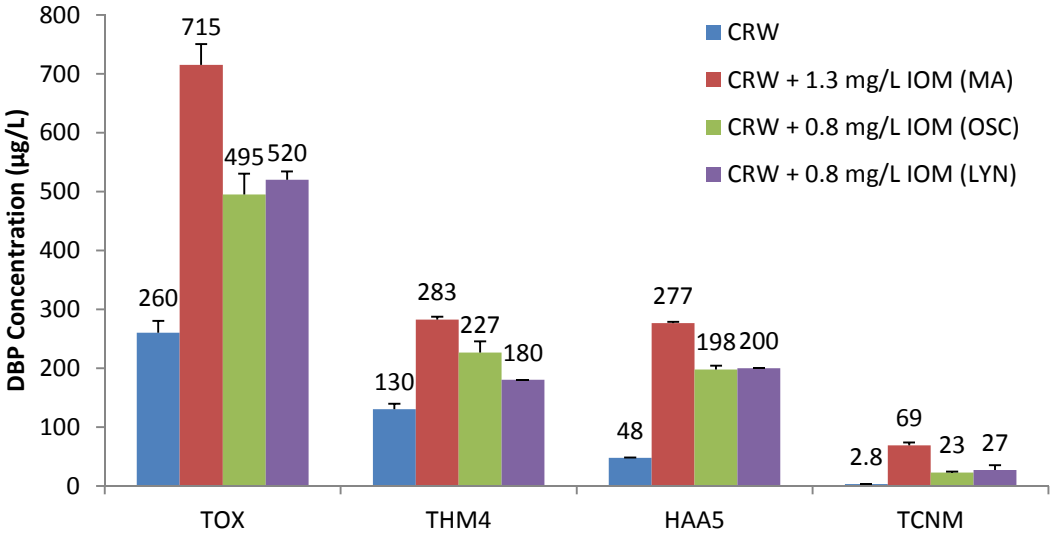

Figure S4. DBP formation after O_3/FCl_2 treatment in CRW (pH=8.25, T=23°C) with the addition of IOM from MA (1.3 mg/L), OSC (0.8 mg/L), and LYN (0.8 mg/L).

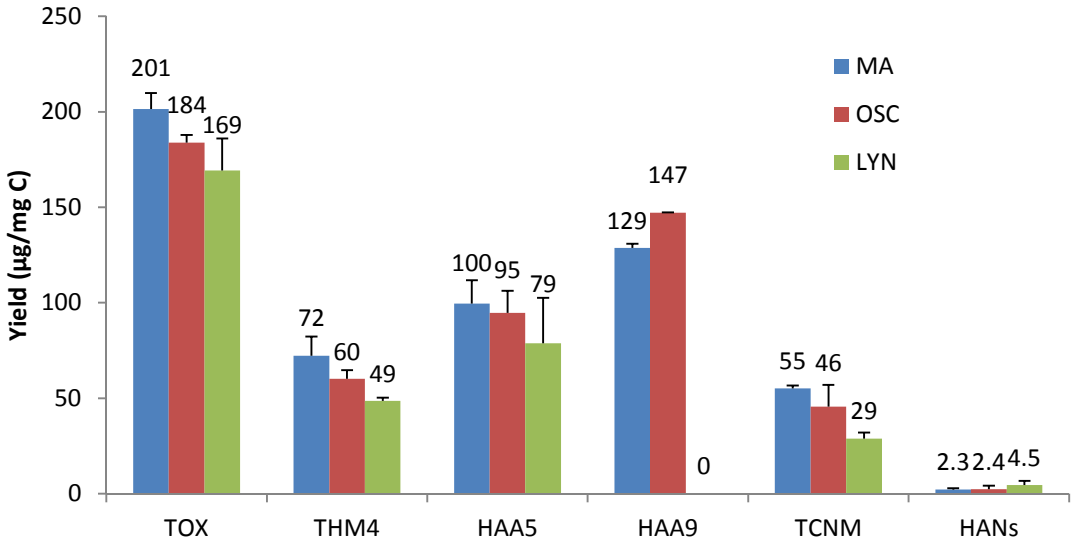

Figure S5. DBP yields from IOM extracts (MA, OSC, and LYN) after FP testing with free chlorine in buffered MQ water (pH=7.5, T=22-24°C, 100 µg/L bromide). (Notes: Error bars indicate +1 RSD, *TOX yield in terms of µg Cl/mg C)

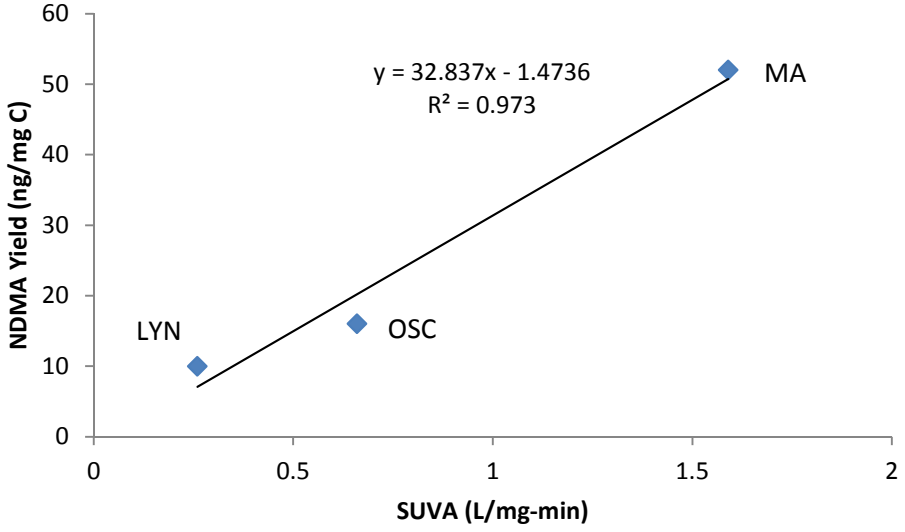

Figure S6. – Relationship between NDMA yield and SUVA for the IOM extracted from MA, OSC, and LYN.