Supplementary Information

Oxidation of Microcystin-LR by Ferrate(VI): Kinetics, Degradation Pathways, and Toxicity Assessments

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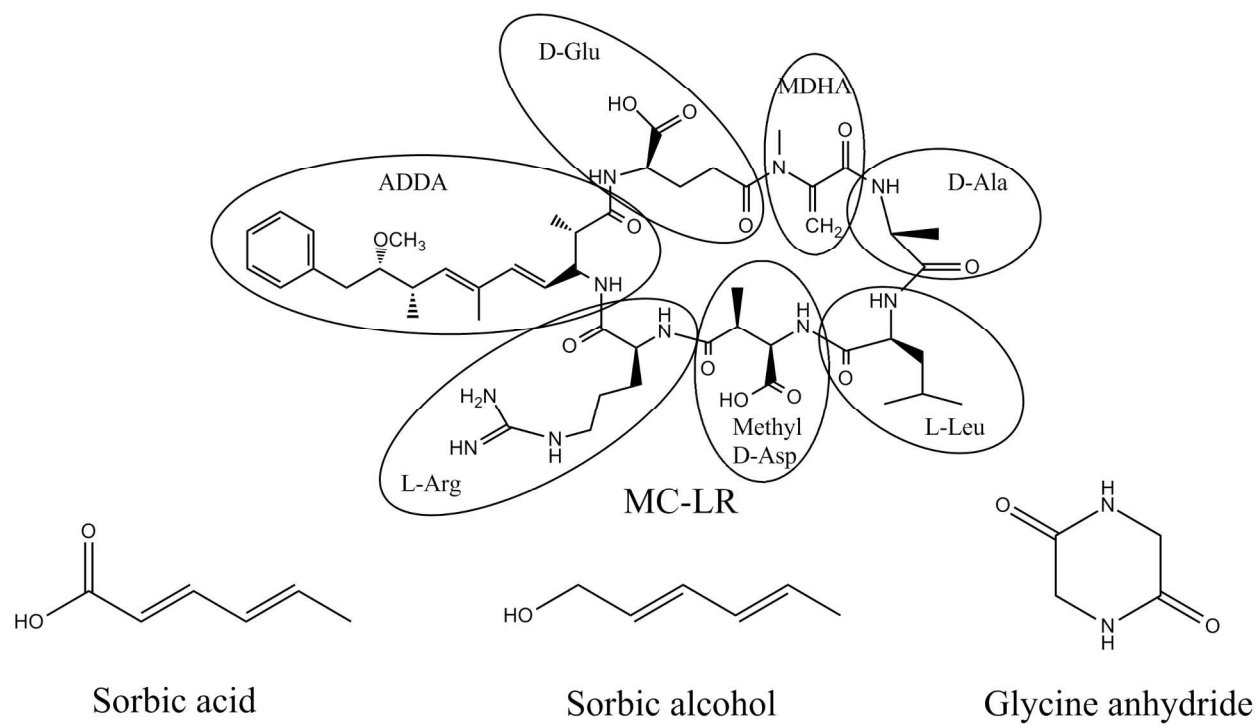Number of Table: 1

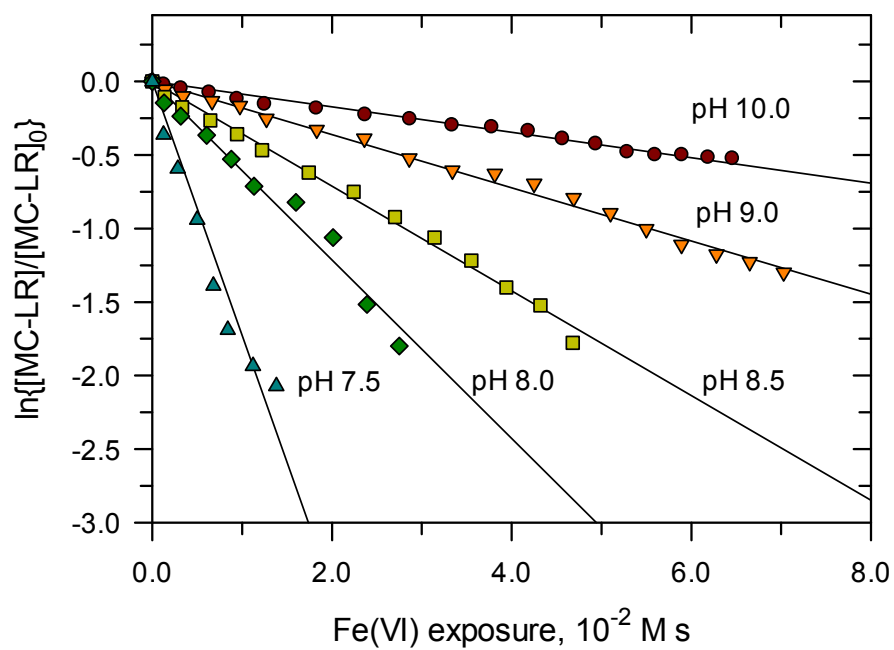Number of figures: 15

References: 1

Text S1

Reagents. All chemicals used in experiments were purchased from either Sigma-Aldrich or Fischer Scientific and were of reagent grade or better. Suwannee river fulvic acid (SRFA) was obtained from the International Humic Substance Society (IHSS). MC-LR was purified from a laboratory culture of *Microcystis aeruginosa* (CCMP299) using a previously described procedure.[1] The purity of MC-LR was > 98 %, confirmed by high performance liquid chromatography (HPLC) method.[1] Solutions were prepared using distilled water passed through an 18MΩ Milli-Q cm water purification system. All solutions were prepared in a 0.01 M sodium phosphate buffer (di or tribasic depending on desired pH). Fe(VI) was prepared at high purity (> 98%) as potassium ferrate (K_2FeO_4).[2] Fe(VI) solutions were prepared by adding solid K_2FeO_4 to a 0.005 M Na_2HPO_4/0.001 M $Na_2B_4O_7 \cdot 10H_2O$ buffer solution at pH 9.00. The concentration of Fe(VI) was determined by measuring the absorbance of the solution at 510 nm ($\varepsilon_{510} = 1150$ M^{-1}cm^{-1}).[2] This approach allowed to quantify the concentrations to low level of 1 μM. The pH of the solutions was adjusted before adding Fe(VI) with the addition of NaOH or H_3PO_4. An accumet pH probe and an Orion 720A pH meter were used to determine the pH of the solution. The pH of the reaction mixtures was determined after mixing substrate and Fe(VI) solutions.

Treatment Studies. Experiments on removing MC-LR were performed in phosphate buffer (0.01 M) and lake water. The lake is located at Brno, Czech Republic. The total organic carbon in the lake water was 7.9 mg L^{-1}. The water samples were spiked separately with 25.0 μg L^{-1} MC-LR and 5.0 mg L^{-1} as FeO_4^{2-} was added into the samples. The mixed solutions were allowed to react for 30 minutes before analyzing for MC-LR in samples. At this reaction time, Fe(VI) and MC-LR were completely degraded. Before analyzing MC-LR by the HPLC technique, samples
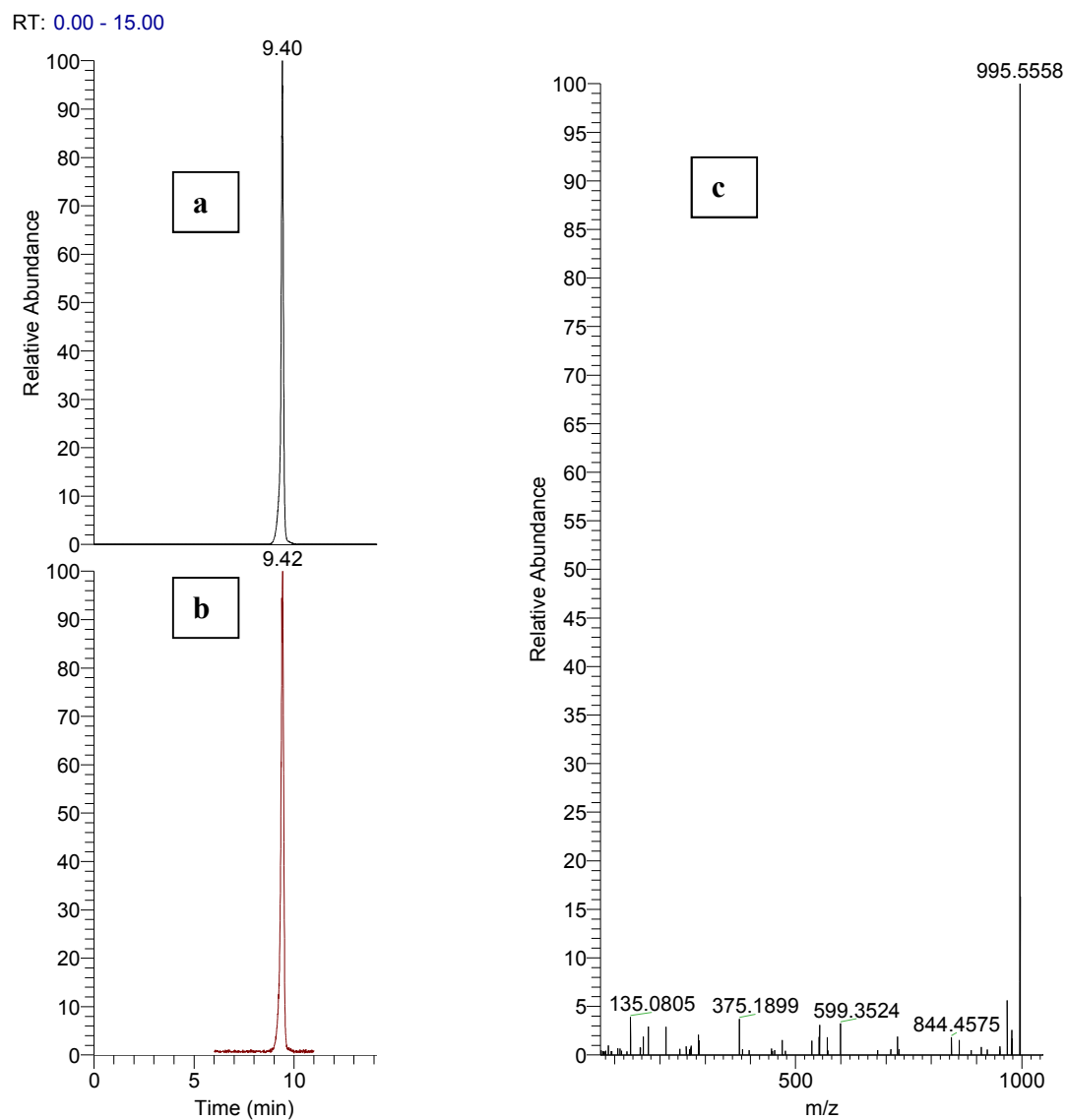
were concentrated by using solid phase extraction method. The extraction was carried out on ODS cartridge (Supelclean LC-18, 3-mL tube, Supelco, Bellefonte, USA). MC-LR was eluted from the cartridge with a methanol/water (90:10, v/v) mixture containing 0.1 % TFA. The eluent was evaporated to dryness at 45 ^{o}C. The solid extract was dissolved in 50 % methanol-water solvent before subjected to analysis. Three replicates were performed at each condition.
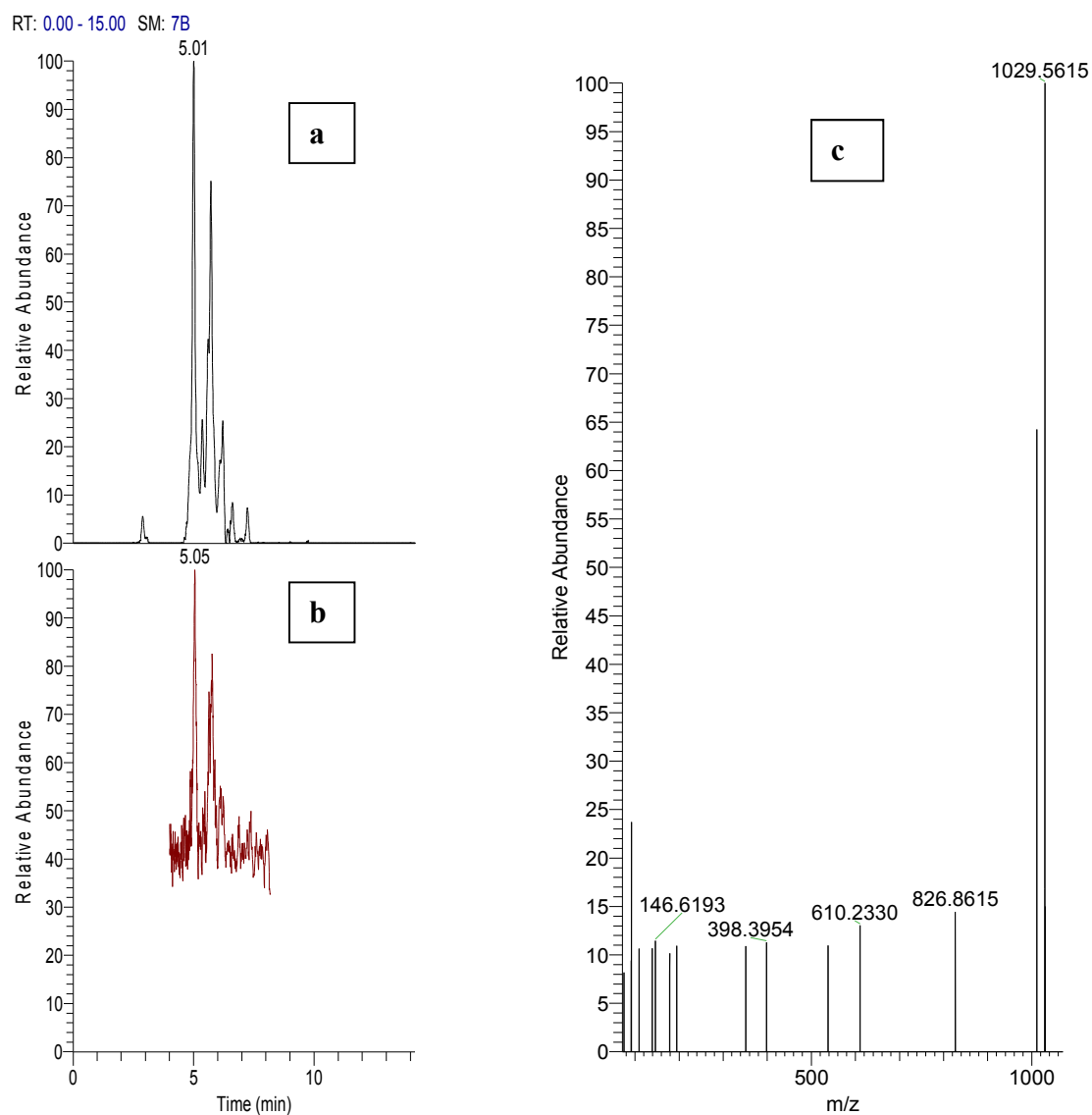
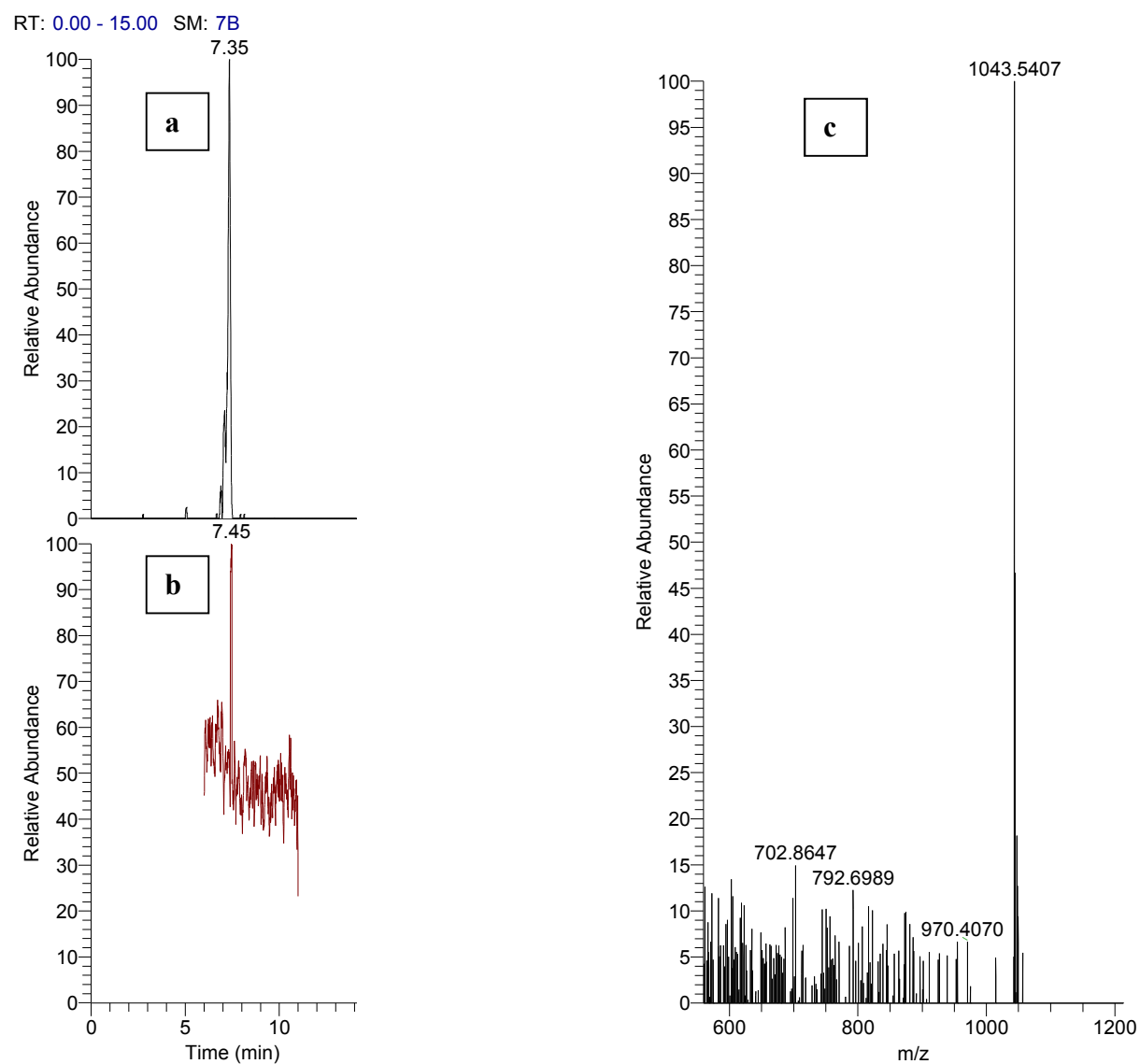**Figure S1.** Structures of microcystin-LR and model compounds.

Figure S2. Fit of kinetic data using eq. (1) for the oxidation of MC-LR by Fe(VI) at 25 $^{\circ}$C. (Experimental conditions: [MC-LR] = 1 $\times 10^{-6}$ M). The data point represent average of three runs at each pH.

Figure S3. (a) LC-MS chromatogram of MC-LR, (b) LC-MS/MS chromatogram of MC-LR, and (c) MS/MS spectra of MC-LR
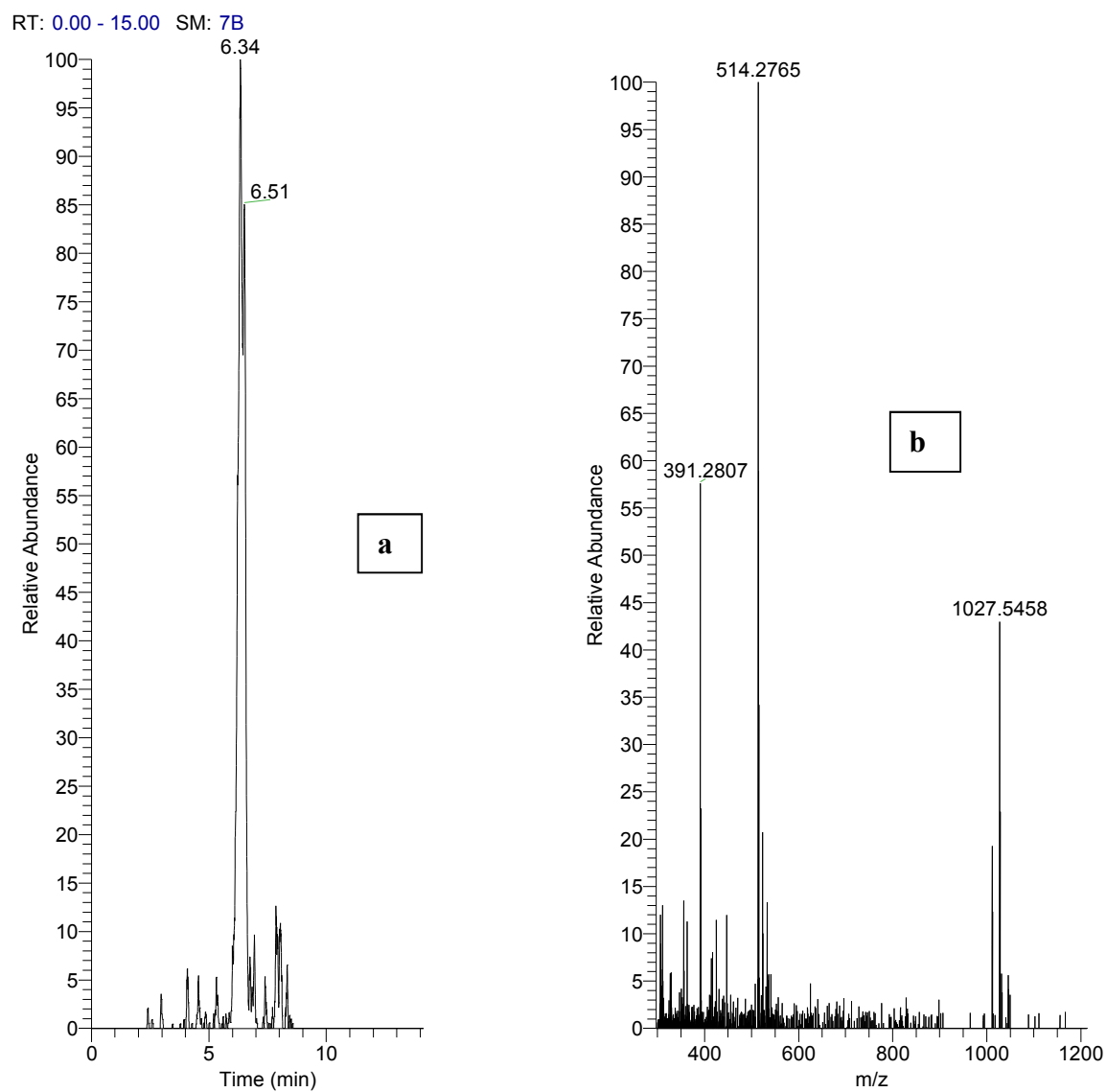
Figure S4. (a) LC-MS chromatogram of OP-1029, (b) LC-MS/MS chromatogram of OP-1029, and (c) MS/MS spectra of OP-1029
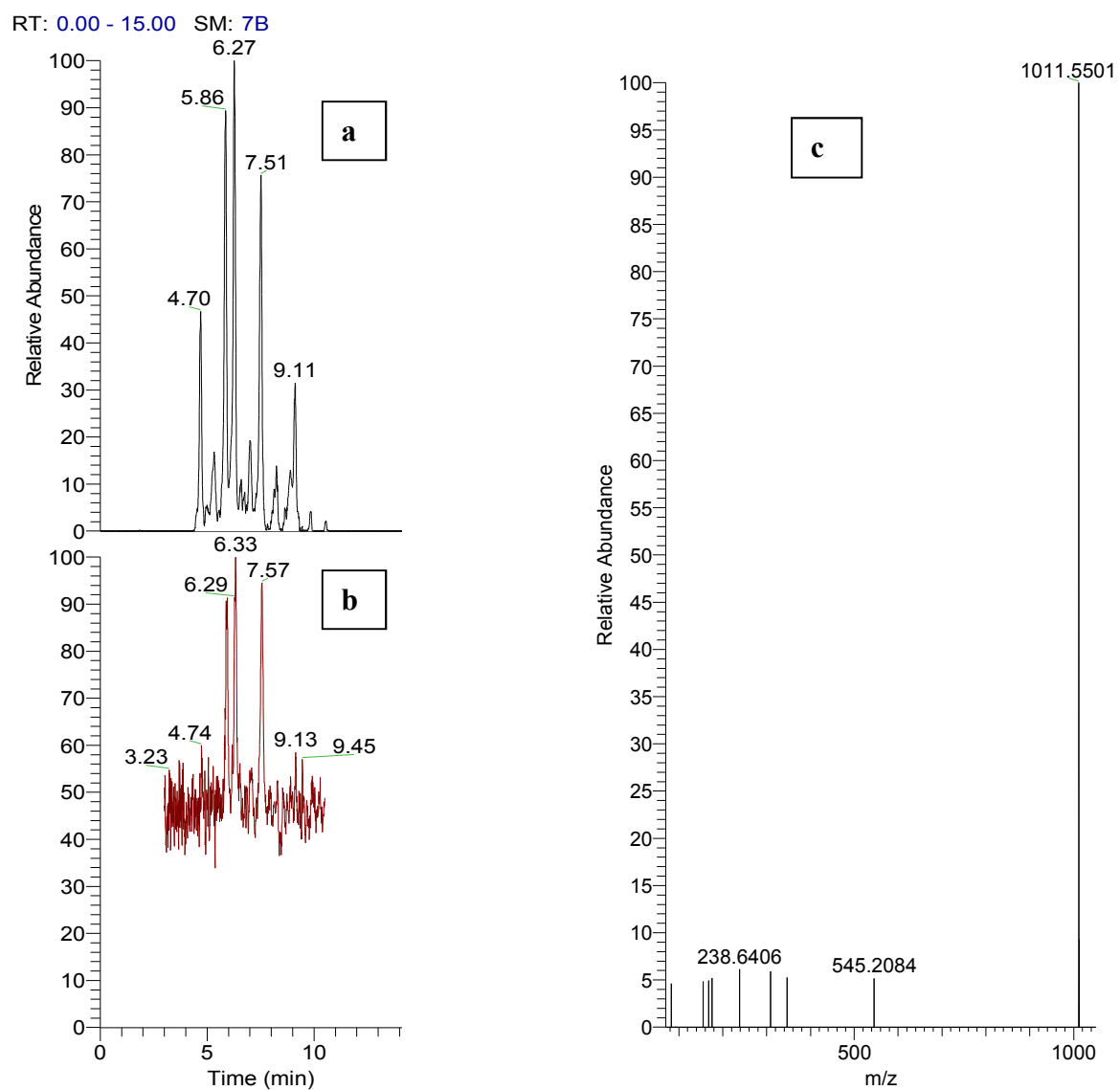
Figure S5. (a) LC-MS chromatogram of OP-1043, (b) LC-MS/MS chromatogram of OP-1043, and (c) MS/MS spectra of OP-1043
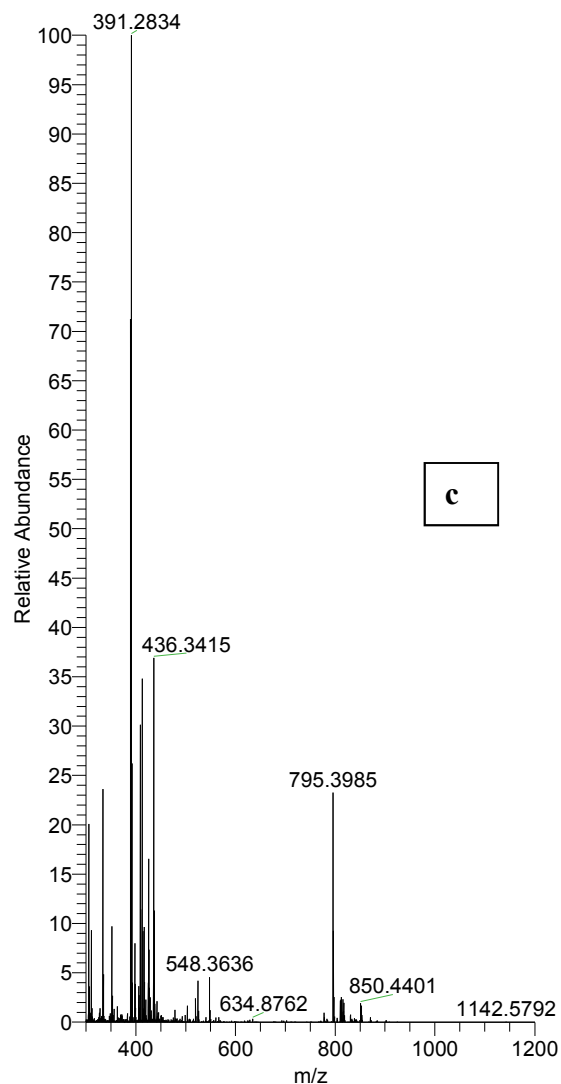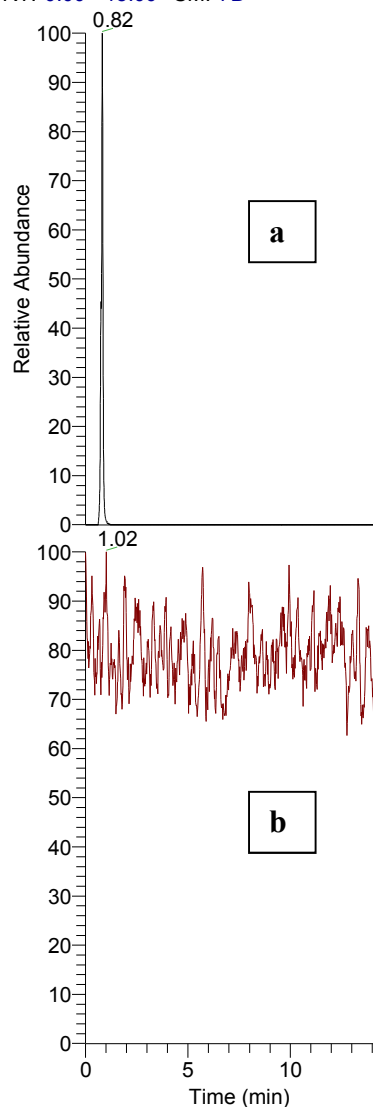
Figure S6. (a) LC-MS chromatogram of OP-1027, and (b) MS/MS spectra of OP-1027 (Note: Intensity of the parent was very low, hence MS2 was not performed)
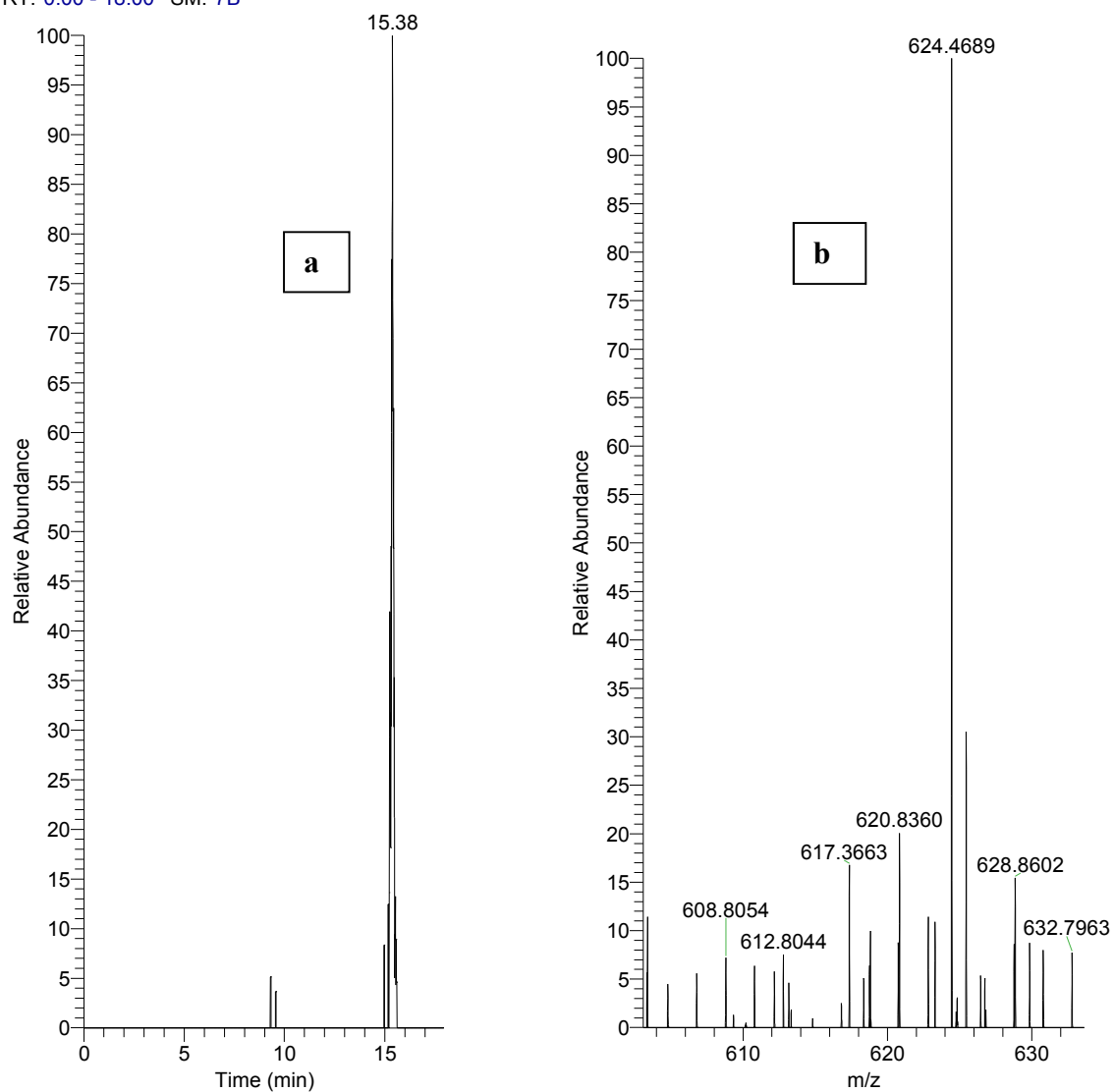
Figure S7. (a) LC-MS chromatogram of OP-1011, (b) LC-MS/MS chromatogram of OP-1011, and (c) MS/MS spectra of OP-1011
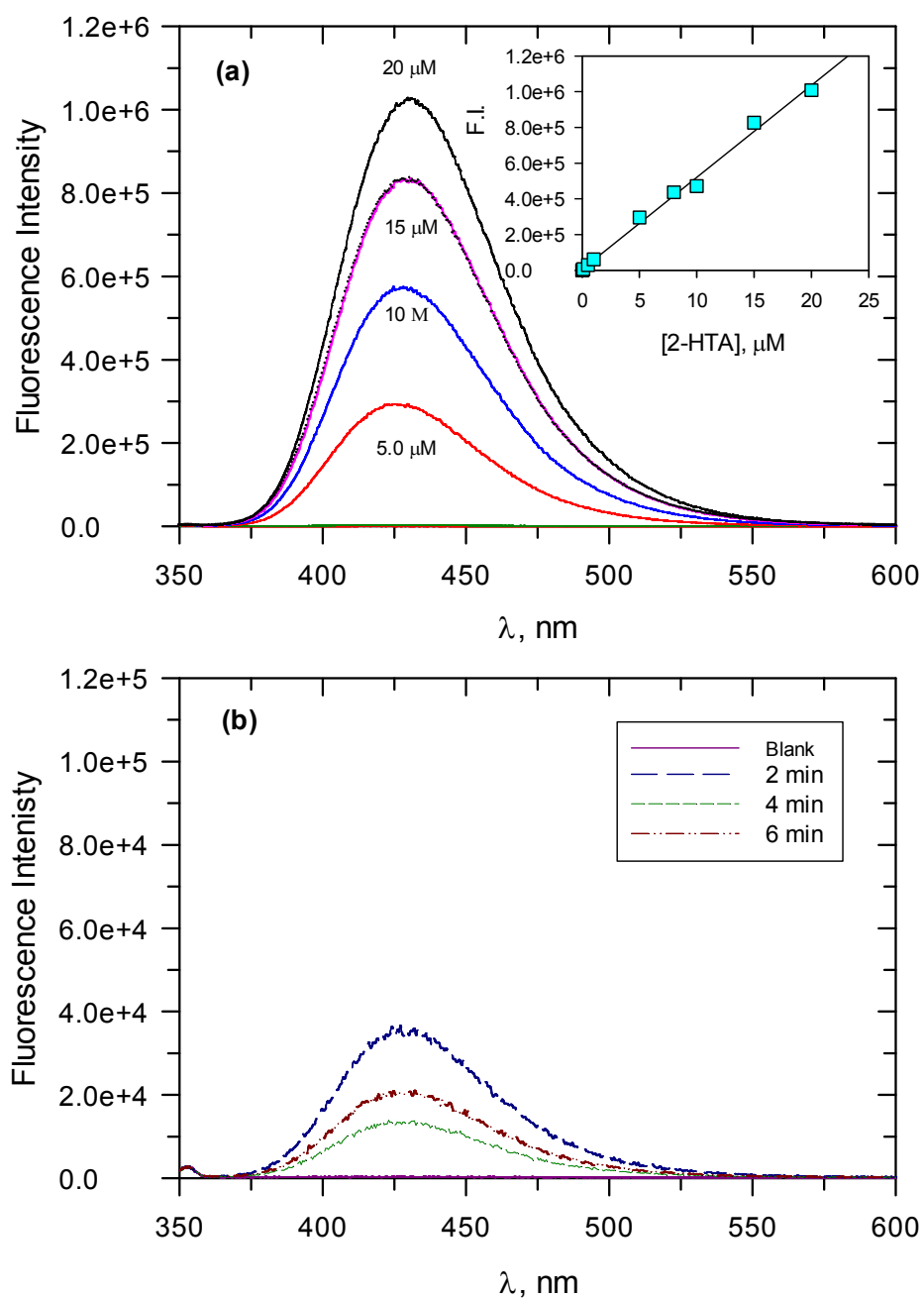
Figure S8. (a) LC-MS chromatogram of OP-795,   (b) LC-MS/MS chromatogram of OP-795, and (c) MS/MS spectra of OP-795 (Note: This product was identified after MS2 experiment was performed)
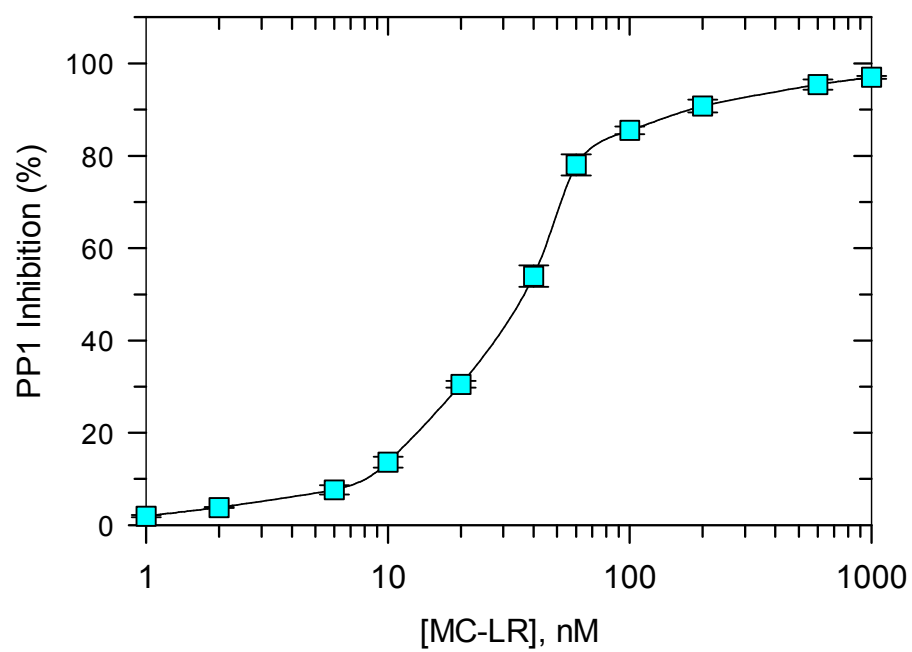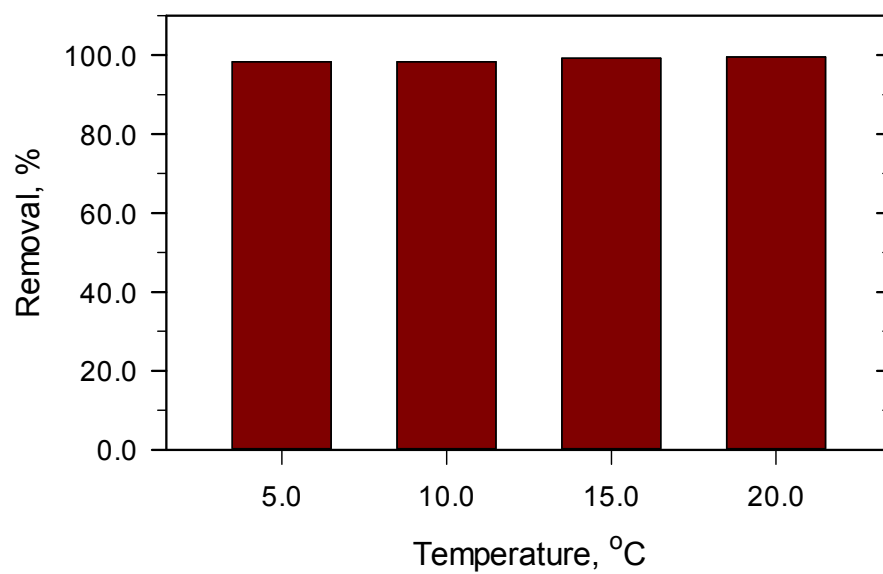
Figure S9. (a) LC-MS chromatogram of OP-617, and (b) MS/MS spectra of OP-617 (Note: Intensity of the parent was very low, hence MS2 was not performed)
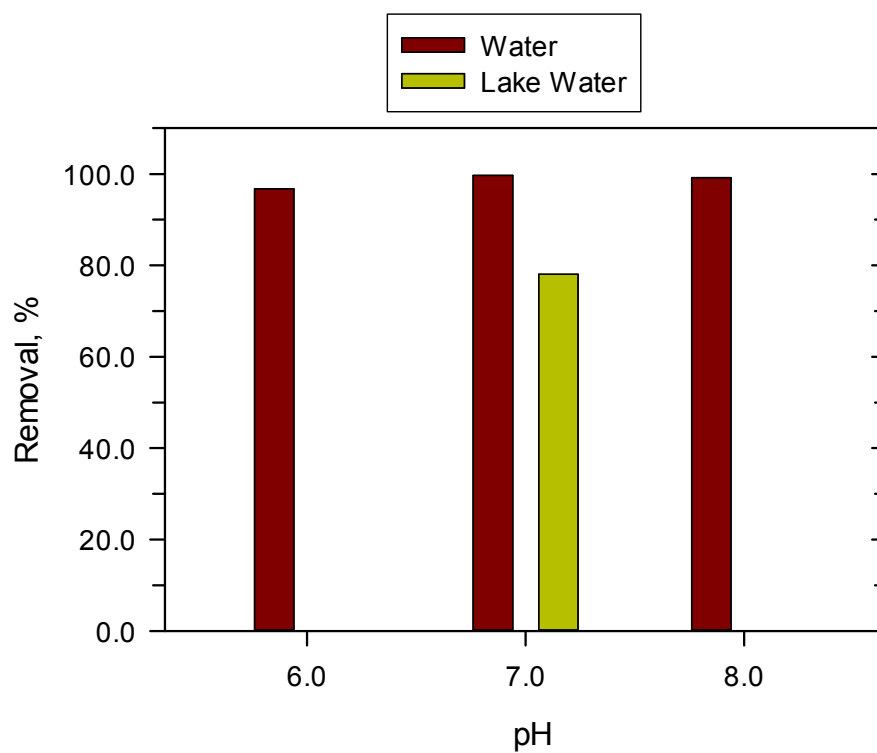
Figure S10. **(a)** Typical spectra and inset calibration curve of 2-hydroxy terephthalate acid (2-HTA) and **(b)** Measurement of $^\bullet$OH using terephthalate acid during Fe(VI) treatment. (Blank = a solution containing TA + quencher; ([Fe(VI)] = 66.6 µM, [MC-LR] = 3.33 µM).

Figure S11. Standard curve of inhibition of PP1 activity by MC-LR
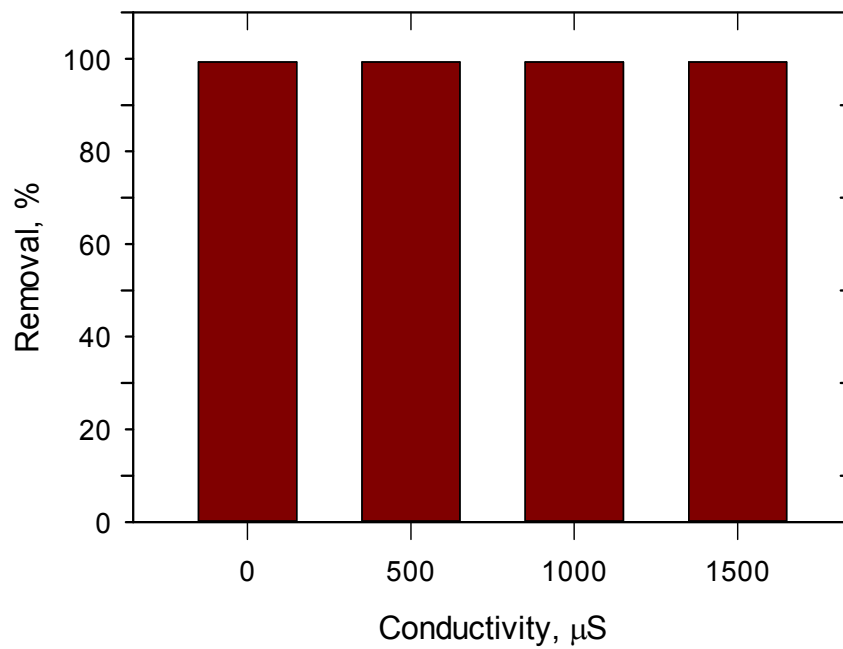
Figure S12. Removal of MC-LR in deionized water by Fe(VI) at different temperatures. ([MC-LR] = 25.0 μg L^{-1}, [FeO$_4$$^{2-}$] = 5.0 mg L^{-1}, pH 7.0**)**

Figure S13 Removal of MC-LR in deionized water and lake water by Fe(VI). ([MC-LR] = 25.0 μg L^{-1}, [FeO$_4$$^{2-}$] = 5.0 mg L^{-1}, and temperature 20 ^{o}C).

Figure S14. Removal of MC-LR in water by Fe(VI) at different concentration of NaHCO$_3$ at 20 $^{\circ}$C. ([MC-LR] = 25.0 μg L^{-1}, [FeO$_4^{2-}$] = 5.0 mg L^{-1}, pH 7.0)

Figure S15 Effect of fulvic acid on the removal of MC-LR by Fe(VI). (Experimental conditions: [Fe(VI)] = 10 µM; [MCLR] = 0.5 µM; pH 9.0)

Table S1. Characteristic of MC-LR and oxidized products (OP).

	Mass	Proposed formula	Theoretical m/z[a]	Experimental m/z[b]	Mass error (ppm)
MC-LR	994.5488	$C_{49}H_{74}N_{10}O_{12}$	995.5560	995.5558	-0.2
OP	1042.5335	$C_{49}H_{74}N_{10}O_{15}$	1043.5408	1043.5407	-0.1
OP	1028.5542	$C_{49}H_{76}N_{10}O_{14}$	1029.5615	1029. 5615	0
OP	1026.5386	$C_{49}H_{74}N_{10}O_{14}$	1027.5459	1027.5458	-0.1
OP	1010.5437	$C_{49}H_{74}N_{10}O_{13}$	1011.5501	1011.5506	0.5
OP	794.3923	$C_{34}H_{54}N_{10}O_{12}$	795.3985	795.4001	2
OP	616.3584	$C_{31}H_{48}N_6O_7$	617.3663	617.3663	0

[a] Calculated using ChemDraw 2010. [b] Observed in Orbitrap mass spectrometer

Table S2. Average concentrations and standard deviation of MC-LR in treatment studies in deionized buffered solution at different pH and temperature ($[FeO_4^{2-}] = 5.0$ mg L^{-1}).

Temperature (°C)	Before Fe(VI) Treatment (µg L^{-1})	After Fe(VI) Treatment (µg L^{-1})			
		pH 6.0	7.0	8.0	9.0
20	25.05±0.04	0.83±0.03	0.08±0.02 (6.21±0.25)[*]	0.43±0.06	6.02±0.13
15			0.20±0.04		
10			0.43±0.04		
5			0.43±0.03		

*Lake Water

References

(1) Song, W.; Teshiba, T.; Rein, K.; O'Shea, K. E. Ultrasonically induced degradation and detoxification of microcystin-LR (Cyanobacterial Toxin). *Environ. Sci. Technol.* **2005**, *39*, 6300-6305.

(2) Luo, Z.; Strouse, M.; Jiang, J. Q.; Sharma, V. K. Methodologies for the analytical determination of ferrate(VI): A Review. *J. Environ. Sci. Health - Part A Toxic/Hazard. Subs. Environ. Eng.* **2011**, *46*, 453-460.