

SUPPORTING INFORMATION SECTION

Algal Uptake of Hydrophobic and Hydrophilic Dissolved Organic Nitrogen in Effluent from Biological Nutrient Removal Municipal Wastewater Treatment Systems

Haizhou Liu[†], Joonseon Jeong^{†‡}, Holly Gray[§], Scott Smith[§], and David L. Sedlak^{*†}

[†] Department of Civil and Environmental Engineering, University of California at
Berkeley, Berkeley, CA 94720 USA

[‡] Current address: Samsung Advanced Institute of Technology, Mt. 14, Nongseo-Dong,
Giheung-Gu, Yongin-Si, Gyeonggi-Do, 446-712, Korea

[§] Department of Chemistry, Wilfrid Laurier University, 75 University Avenue West
Waterloo, ON, N2L 3C5 Canada

* Corresponding author, e-mail: sedlak@berkeley.edu, phone (510) 643-0256

Submitted to Environmental Science and Technology

Table of Contents

Procedure to clean XAD-8 resin	S3
Ion exchange treatment	S3
Preparation of algal inoculum	S4
Table S1 Details of the type of treatment and disinfection in each wastewater treatment plant.....	S6
Table S2 Relative fluorophore concentrations of hydrophobic and hydrophilic fractions of wastewater effluents.....	S7
Figure S1 Dissolved organic carbon concentrations in the hydrophobic and hydrophilic extracts from all wastewater effluent samples investigated in this study.	S8
Figure S2 Changes of DON and DOC concentrations before and after ion exchange (IEX) resin treatment of XAD-8 hydrophilic extract from all wastewater effluent samples.	S9
Figure S3 Bioavailability of XAD-8 resin extracts of King William Wastewater Treatment Plant (KWWTP) effluent in 14-day algal bioassay experiments.	S10
Figure S4 Bioavailability of the hydrophobic fraction of Truckee Meadows Wastewater Treatment Plant (TMWTP) effluent spiked with 0.7 mg N/L NO_3^- during the 14-day algal bioassay.....	S11
Figure S5 Corrected emission-excitation fluorescence of hydrophobic and hydrophilic fractions of wastewater effluents..	S12
Figure S6 Percentage of DON consumed in hydrophobic and hydrophilic extracts during 14-day algal bioassays.....	S13

Procedure to clean XAD-8 resin

A batch of 5g XAD-8 resin was suspended in approximately 50 mL of 0.1M NaOH solution. After 24 hours, the NaOH solution was decanted and replaced. This step was repeated for 3 consecutive days. After the final rinse, the resin was stored in methanol until use. To conduct fractionation experiments, 5g clean resin was first suspended in 50 mL of deionized water, then packed in a borosilicate glass column (Flex-Column, 1.0 cm diameter, 10 cm length, Thomas Scientific, Swedesboro, NJ). The bed volume of the resin in the column was 5 mL. A threaded flow adapter was also installed to eliminate any dead space in the column. The column was rinsed with approximately 3L of deionized water until the dissolved organic carbon (DOC) concentration in the effluent was less than 0.2 mg C/L. The packed column was then rinsed with 100 bed volumes of 0.1 M NaOH followed by 100 bed volumes of 0.1 M HCl, alternating between acid and base three times, and followed by 100 bed volumes of deionized water. The DOC concentration of the final deionized water wash solution coming out of the clean resin column was tested to verify that it was less than 0.2 mg C/L. One column of XAD-8 resin was used to treat 400 mL of samples and was not reused. Measurements of DOC concentration in aliquots of effluent ensured no column breakthrough.

Ion exchange treatment

1 g of the anion exchange resin (Dowex 1X8 chloride form resin, Sigma-Aldrich) was cleaned by rinsing with 20 mL of 3 M NaCl followed by 100 mL of deionized water. After cleaning, the resin was packed in a borosilicate glass column (FlexColumn, 1.0 cm diameter, 10 cm length, Thomas Scientific).

A series of parameters, including resin mass loading, flow rate, pH of eluent, affect the efficiency of ion exchange treatment. Details on the optimization of ion exchange treatment are described in a recent WERF report (1). The optimized approach includes using 1 g of resin to treat 100 mL of hydrophilic fraction that was acidified to pH 2.0, controlling the flow rate at 5 mL/min and treating the sample with ion exchange resin after XAD-8 resin separation. This operational condition ensured no breakthrough of NO_3^- and minimal DON adsorption on ion exchange resin.

Preparation of algal inoculum

Preparation of the media solution followed the standard EPA method 1003.0 (2). Specifically, the culture medium consisted of 40 mg/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 25.5 mg/L NaNO_3 , 12.2 mg/L $\text{MgCl}_2 \cdot 7\text{H}_2\text{O}$, 14.7 mg/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.04 mg/L K_2HPO_4 , 15 mg/L NaHCO_3 , 300 $\mu\text{g/L}$ $\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$, 185 $\mu\text{g/L}$ H_3BO_3 , 160 $\mu\text{g/L}$ $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 7.26 $\mu\text{g/L}$ $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 3.27 $\mu\text{g/L}$ $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 3 $\mu\text{g/L}$ ZnCl_2 , 2.39 $\mu\text{g/L}$ Na_2SeO_4 , 1.43 $\mu\text{g/L}$ $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ and 0.012 $\mu\text{g/L}$ $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$. The medium pH was adjusted to 7.0 with either 0.01 M HCl or NaOH.

Upon receipt of the *Selenastrum capricornutum* starter culture from University of Texas at Austin, a stock culture was initiated by aseptically transferring 1 mL of the starter culture to a 125 mL Erlenmeyer flask containing 60 mL of medium prepared as above. This algal stock was maintained on a shaker at 25 ± 2 °C using a 12-hour light/dark cycle for one week until the algae were growing in a logarithmic phase and the cell density was high. At the end of each week, 1 mL of stock culture was transferred again to another 60 mL of new medium to keep a continuous supply of healthy cells. The morphology of algal cells was

examined with a microscope periodically to ensure the absence of contaminating microorganisms.

The algal inoculum was prepared within one hour prior of the initiation of the bioassay test. 50 mL of the stock culture that had grown for 7 days was harvested and washed first by centrifuging 10 min at 5000 g. The supernatant was decanted and 50 mL of deionized water was added. This procedure was repeated twice in order to remove residual nitrogen from the algal stock. The washed stock culture was resuspended in a requisite amount of deionized water to yield a final *in vivo* chlorophyll-a concentration of 800 µg/L. New inoculum was prepared every time before the start of a new bioassay test.

Table S1 Details of the type of treatment and disinfection in each wastewater treatment plant.

Wastewater Treatment Facility	Types of BNR Treatment	Solids Retention Time (Day)	Type of Disinfection
Truckee Meadows Water Reclamation Facility	High rate activated sludge with trickling filter and denitrification fluidized bed (methanol feed). Phosphorus removal achieved.	2.5	Chlorination/Dechlorination
Broad Run Water Reclamation Facility	5-stage Bardenpho with membrane bioreactor and granular activated carbon filtration. Phosphorus removal enhanced with alum addition.	19-23	Ultraviolet
HRSD King William Wastewater Treatment Plant	4-stage Bardenpho with membrane bioreactor	23-27	Ultraviolet
San Jose Water Pollution Control Plant	Activated sludge with tertiary nitrification unit and effluent filtration.	8.5-10.5	Chlorination/Dechlorination
North Durham Water Reclamation Facilities	5-stage Bardenpho with nitrified recycle and denitrification filter (methanol feed). Phosphorus removal achieved.	20	Ultraviolet
South Durham Water Reclamation Facilities	5-stage Bardenpho with nitrified recycle and denitrification filter (methanol feed). Phosphorus removal achieved.	16	Ultraviolet
RWSA Moores Creek Wastewater Treatment Plant	5-stage Bardenpho with denitrification filter (methanol addition). Phosphorus removal achieved.	9-14	Ultraviolet
HRSD Nansemond Wastewater Treatment Plant	Original: 3-Stage VIP; Upgraded (December 2010): 5-stage Bardenpho with denitrification filter (methanol feed). P removal achieved	15	Chlorination/Dechlorination

Table S2 Relative fluorophore concentrations of hydrophobic and hydrophilic fractions of wastewater effluents. Fluorophores include Humic Substances, Tyrosine and Tryptophan.

Sample	Relative Fluorophores Concentrations*		
	Humic Substances	Tyrosine	Tryptophan
NDWRF Hydrophobic	0.836	0.121	0.070
NDWRF Hydrophilic	0.334	0.046	0.087
SDWRF Hydrophobic	0.579	0.111	0.069
SDWRF Hydrophilic	0.302	0.041	0.080
RVWRF Hydrophobic	0.584	0.132	0.067
RVWTP Hydrophilic	0.374	0.061	0.074
NAWTP Hydrophobic	0.678	0.131	0.084

* The concentrations of each component are given in relative fluorescence units based on PARAFAC analysis of the corrected fluorescence spectra. The relative fluorescence concentration is linearly proportional to the actual concentration.

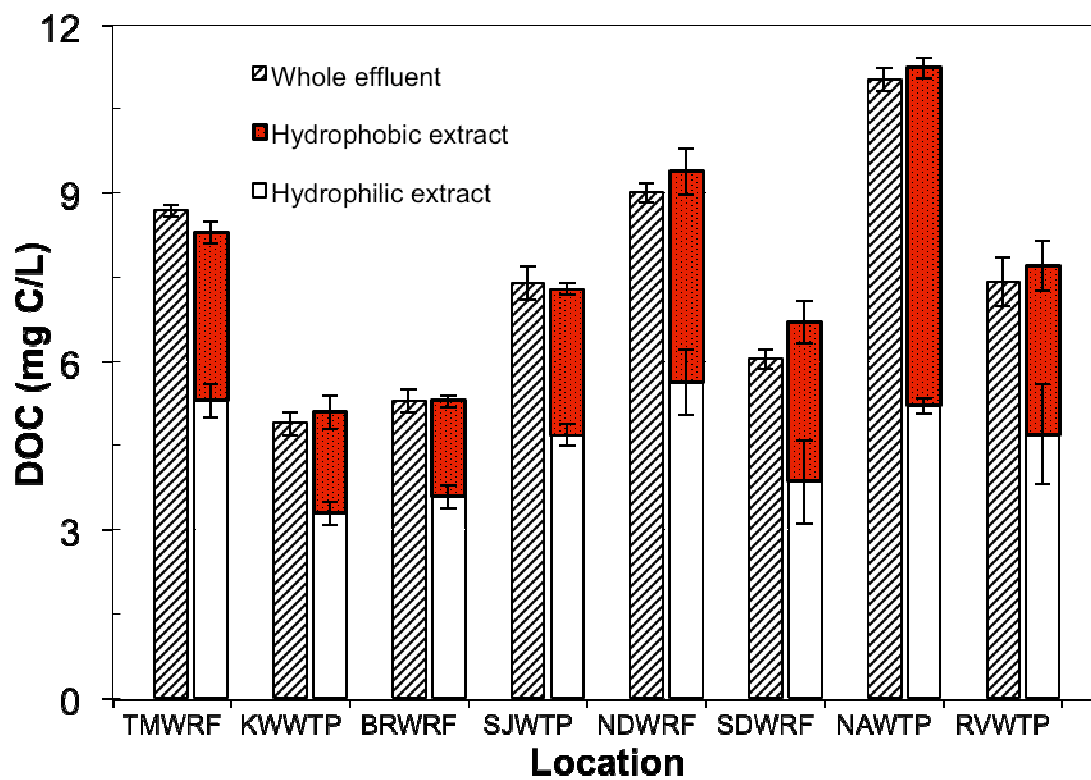


Figure S1 Dissolved organic carbon concentrations in the hydrophobic and hydrophilic extracts from all wastewater effluent samples investigated in this study.

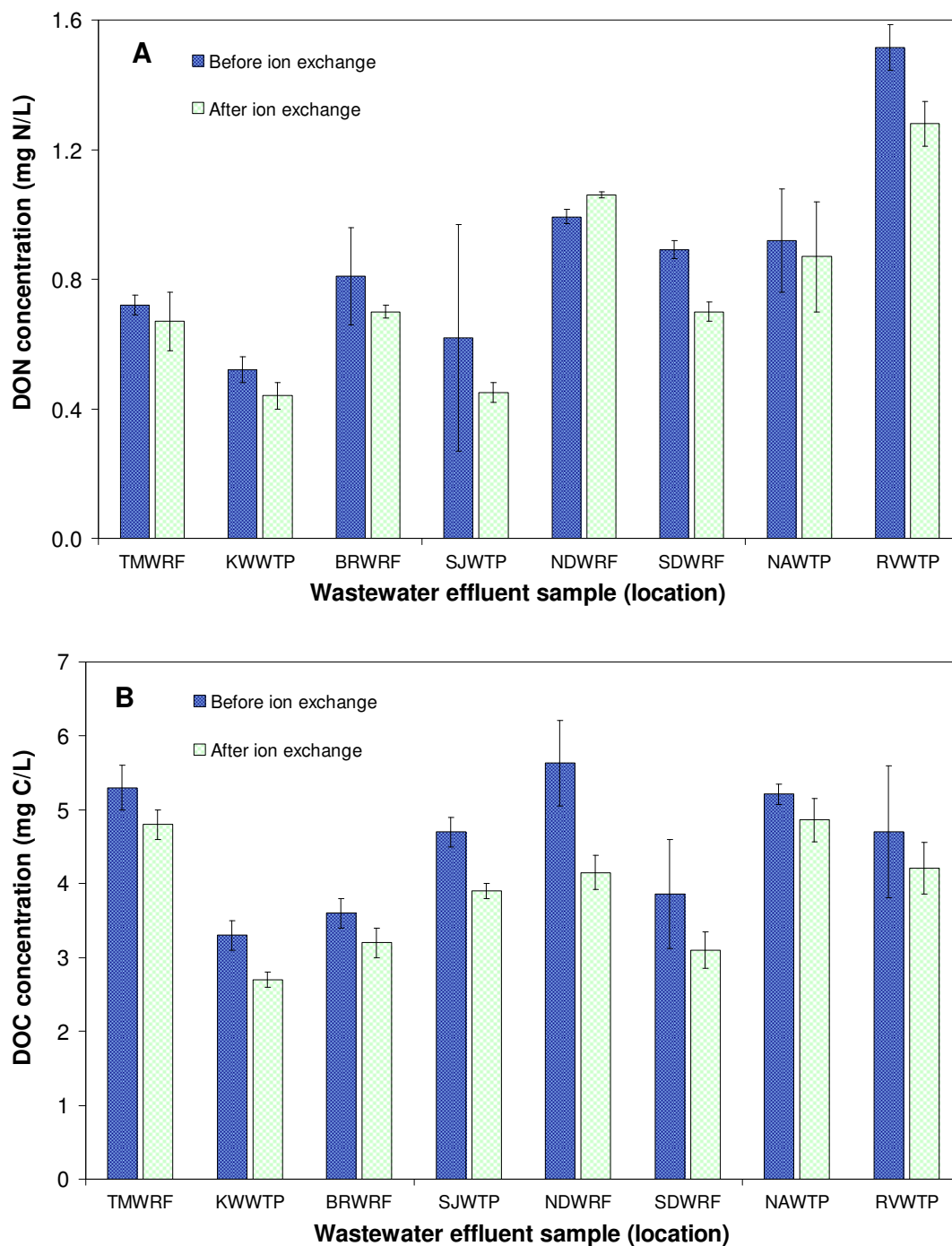


Figure S2 Changes of DON and DOC concentrations before and after ion exchange (IEX) resin treatment of XAD-8 hydrophilic extract from all wastewater effluent samples. (A) DON concentrations; (B) DOC concentrations.

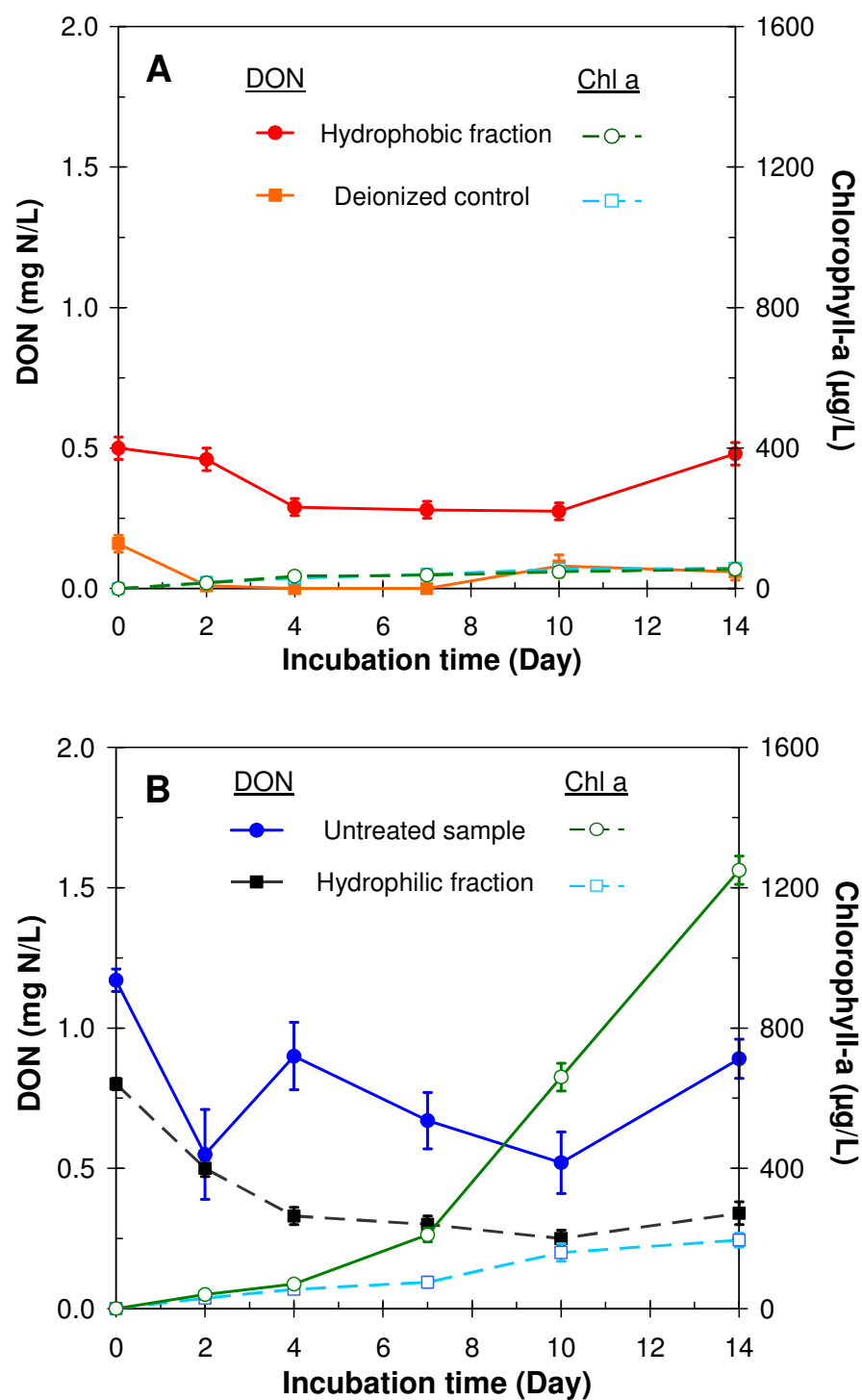


Figure S3 Bioavailability of XAD-8 resin extracts of King William Wastewater Treatment Plant (KWWTP) effluent in 14-day algal bioassay experiments. (A) Hydrophobic fraction and deionized control. The initial hydrophobic DON concentration was four times higher than that detected in the effluent sample; (B) Hydrophilic fraction and the whole untreated effluent.

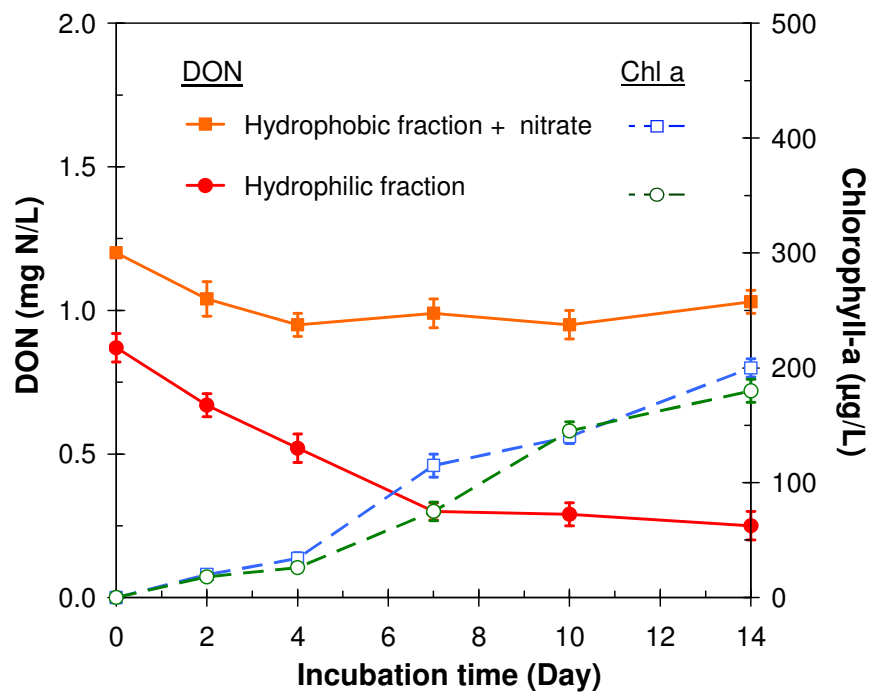


Figure S4 Bioavailability of the hydrophobic fraction of Truckee Meadows Wastewater Treatment Plant (TMWTP) effluent spiked with 0.7 mg N/L NO_3^- during the 14-day algal bioassay. All NO_3^- was consumed after 10 days. The DON concentration in the hydrophilic fraction of the TMWTP effluent is the same as 0.7 mg N/L.

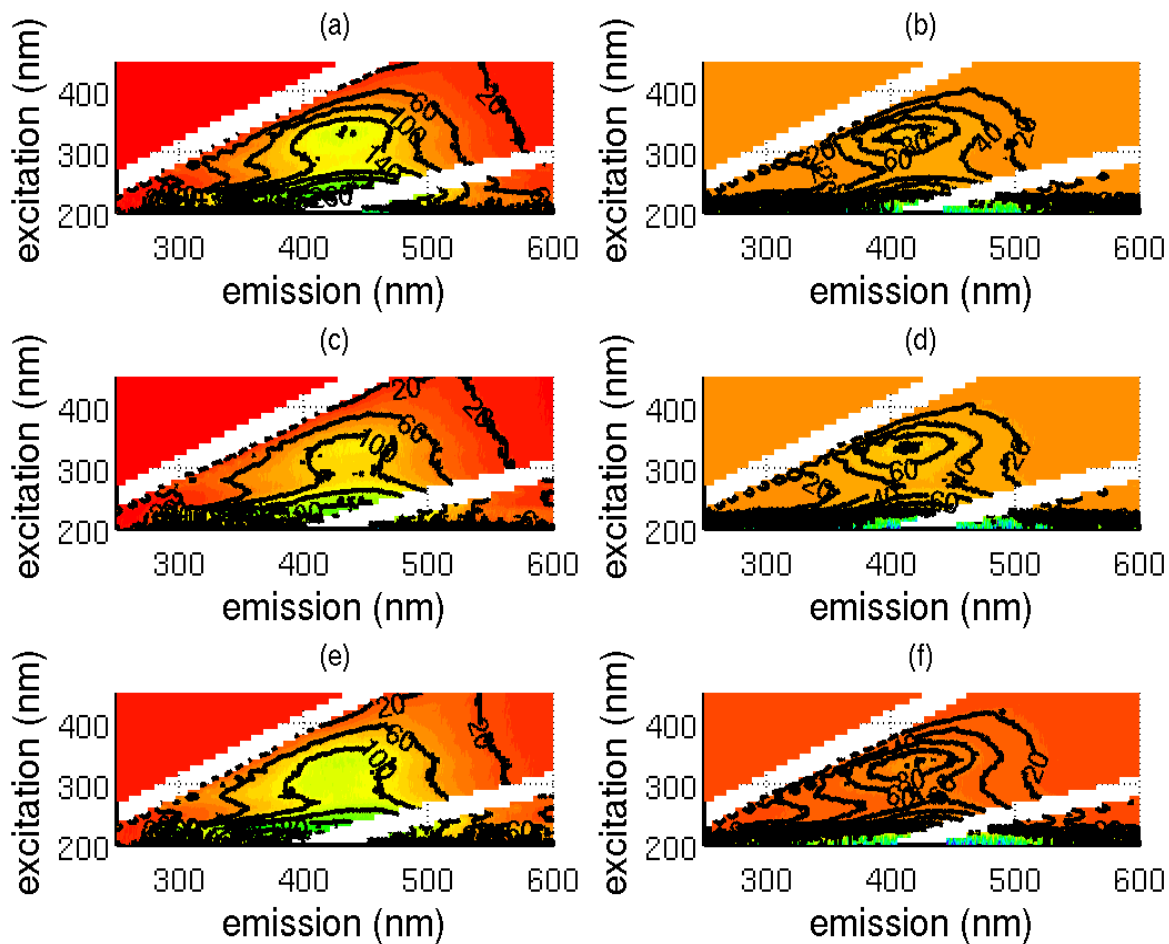


Figure S5 Corrected emission-excitation fluorescence of hydrophobic and hydrophilic fractions of wastewater effluents. (A) NDWRF hydrophobic fraction; (B) NDWRF hydrophilic fraction; (C) SDWRF hydrophobic; (D) SDWRF hydrophilic; (E) RVWTP hydrophobic; (F) RVWTP hydrophilic.

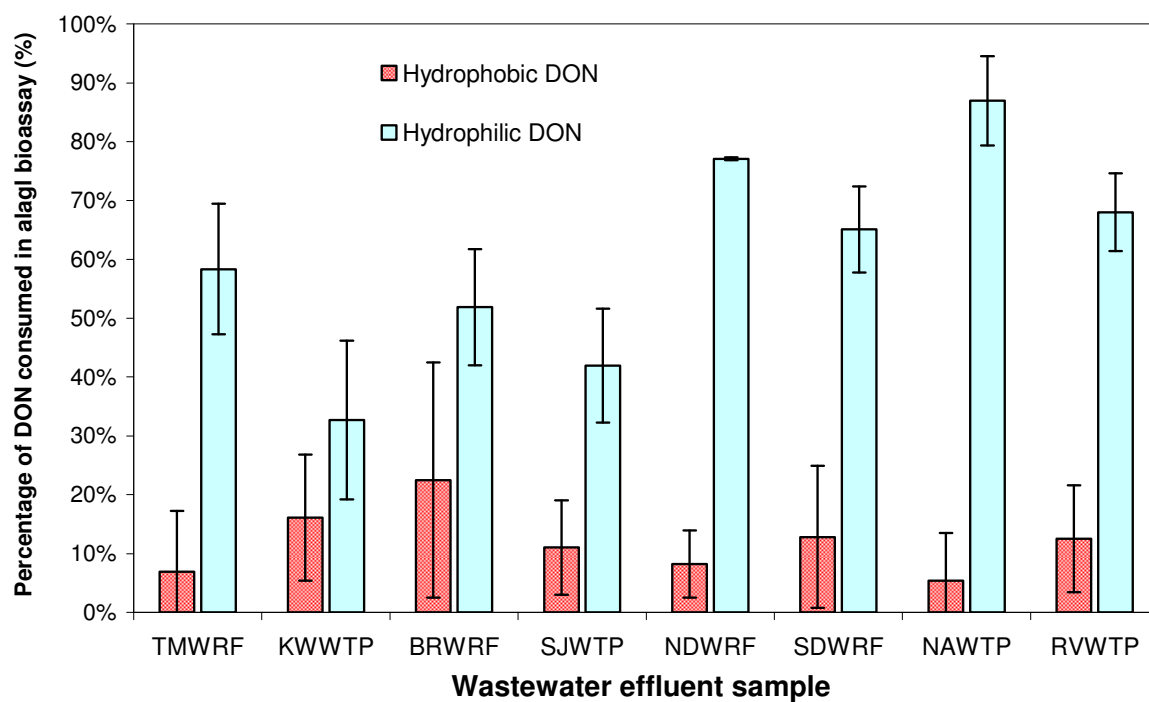


Figure S6 Percentage of DON consumed in hydrophobic and hydrophilic extracts during 14-day algal bioassays.

References

- 1 Sedlak, D.L. Inert fractions of dissolved organic nitrogen in BNR treatment plant effluents. Interim report to the *Water Environment Research Foundation*, 2010.
- 2 United States Environmental Protection Agency. Method 1003.0 in *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms*, 4th edition. Washington DC. 2002.