The fates of aromatic protein and soluble microbial products-like organics, as the precursors of dichloroacetonitrile and dichloroacetamide, in drinking water advanced treatment process

Tao Lin [a, b,*], Han Chen [a, b], Shaoxin Ding [a, b], Wei Chen [a, b], Hang Xu

[a] Ministry of Education Key Laboratory o f Integrated Regulation and Resource Development on Shallow Lakes, Hohai University, Nanjing 210098, PR China

[b] College of Environment, Hohai University, Nanjing 210098, PR China

(* Corresponding author: Email:hit_lintao@163.com; Fax: +86 02583787134; Tel: +86 13951690290)

Text S1 Fluorescence Regional Integration

Fluorescence Regional Integration (FRI) has been successfully applied to the analysis of Fluorescence EEM spectra of water samples. The integration of each fluorescence region was calculated according to Eq. (1) and the result is shown in Table S2.

$$\Phi_{i,n} = MF_i \times \Phi_i = MF_i \int_{ex}\int_{em} I(\lambda_{ex}\lambda_{em}) d\lambda_{ex} d\lambda_{em} \quad (1)$$

where MF_i represents multiple repeatability factor for the region "*i*". I ($\lambda_{ex}\lambda_{em}$) represents the fluorescence intensity corresponding to each excitation-wavelength pair. $\Delta\lambda_{ex}$ represents excitation wavelength increment. $\Delta\lambda_{em}$ represents emission wavelength increment. Both $\Delta\lambda_{ex}$ and $\Delta\lambda_{em}$ are taken as 2 nm in this study.

Text S2 Detection of DCAN and DCAcAm

DCAN and DCAcAm were extracted into methyl tert-butyl ether (MTBE) and Ethyl acetate (ETAC) respectively. They were measured using an gas chromatography (GC, Agilent 7890B, USA) coupled with a micro-electron capture detector (μ-ECD) referred to USEPA Method 551.1 (Munch and Hautman, 1995) and adopted from a published paper with modifications (Yu et al., 2015). To increase extraction rate, 10 g of anhydrous sodium sulfate was added to 100 mL chlorinated water samples placed in 150 mL screw cap vials before liquid-liquid extraction. 10 mL of MTBE was added into the vial using pipette, then vibrated with a vortex mixer (KS501, IKA,

Germany) for 3-4 min, after that let them stand for 10 min for phase separation. Subsequently, the extracts were pipetted and concentrated to 1 mL by a nitrogen evaporator at 37 ℃ and then transferred to GC vials for analysis. N-DBPs were separated by HP-5 column (30 m × 0.25 mm × 0.1 μm, Agilent, USA). The temperature of injection port was set at 235 ℃ and μ-ECD temperature was set at 250℃. In the initial stage, the oven temperature was set to 80 ℃ hold for 5 min, then increased to 150 ℃ at rate of 40 ℃/min and then maintained for 3min and 5 min respectively. The sample injection volume was 2 μL with splitless liner. The detection limits of DCAN and DCAcAm were 15.6 and 13.2 ng/L respectively.

Text S3 Measurement of amino acids and proteins

For the analysis of amino acids, according to a method modified from the paper by Dotson and Westerhoff (2009) and Martens and Loeffelmann (2003), liquid phase 4 N methanesulfonic acid was added to 1000μL of water samples at 135℃ for 90 min to hydrolyze. Centrifuge the hydrolysate, 200μL of the supernatant was collected and freeze centrifuged using High Speed Tabletop Refrigerated Centrifuge (Hettich, Mikro 220R, Germany), then they were concentrated and dried at 40 ℃ under argon with a Reacti-Therm heating module (Pierce, USA). The residue was redissolved with 200 μL of acetonitrile : water = 1:1 solution and then analyzed with a High Performance Liquid Chromatograph (HPLC, Shimadzu LC-20AD, Japan) coupled with a tandem mass spectrometry (AB Sciex, QTrap5500, America) following the procedures developed by Cohen and Dennis (1993) standards of amino acid were a hydrolyzed protein amino acid mixture produced by Pierce (USA), which contained 17 individual primary amino acids. The operation condition of HPLC was as followed: the BEH Amide column (100 mm × 2.1 mm, 1.7 μm) was used to separate amino acids, the temperature of column was set to 50 ℃, Mobile phase A was Milli-Q water mixed with 0.1% formic acid, mobile phase B was acetonitrile mixed with 5 mmol/L of ammonium acetate and 0.1% formic acid, Sample injection was 1 μL.

Proteins were determined according to a modified Lowry method (Frølund et al., 1995). Bovine serum albumin solution at a concentration of about 250 mg/ml was used for standard protein solution. The absorbance of the solution was measured at 700 nm by a HITACHI U-3900H spectrophotometer.

Text S4 High-throughput sequencing analysis

About 20 g activated carbon granules were sampled from the BAC filter and then stored at 4 ℃ until analysis. DNA from biofilm attached to activated carbon granules was extracted as described in previous studies (Boon et al., 2000; Lautenschlager et al., 2014), using the E.Z.N.A Mag-Bind Soil DNA Kit (Omega, USA). The preprocessed sequences were clustered into Operational Taxonomic Units (OTUs) with a minimum identity of 97% by UCLUST software, then they were used to estimate relative abundance of bacterial community. The taxonomic compositions of bacterial communities at phylum, class, order, family and genus level were analyzed respectively. The diversity indexes such as Equitability, Shannon and Simpson were calculated by QIIME pipeline as described in a published study (Telias et al., 2011).

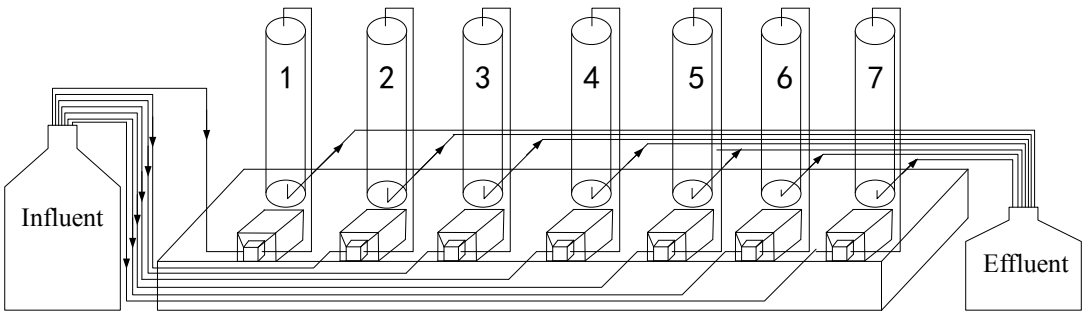
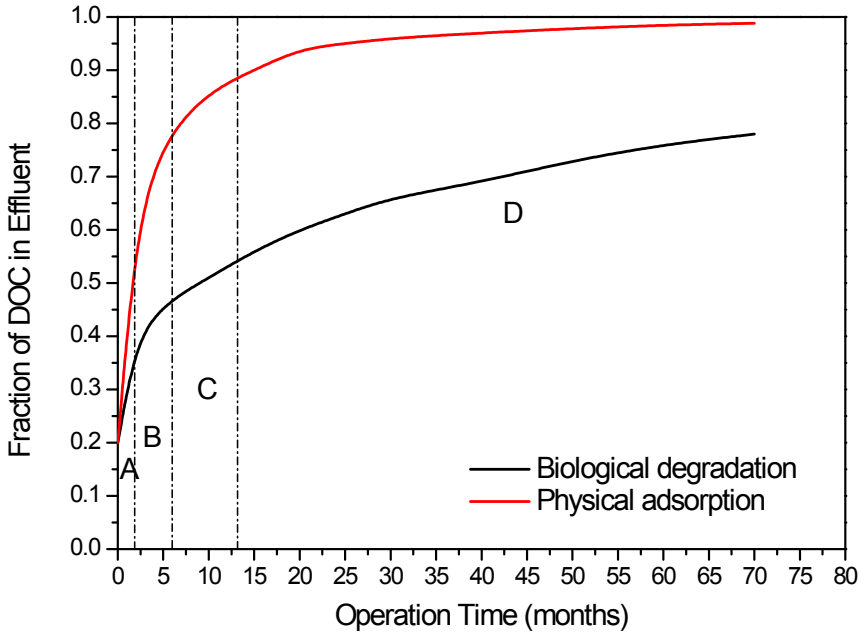


Fig. S1 Bench-scale BAC filters



78

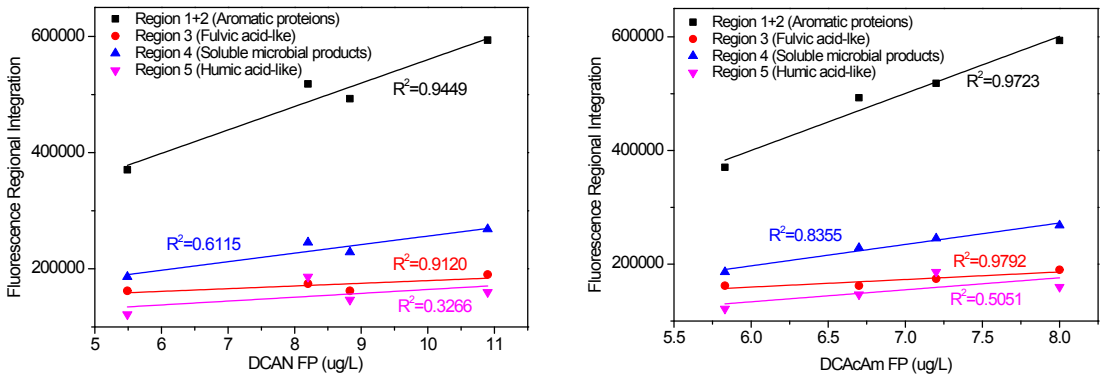
79 **Fig. S2** Conceptualized representation of ozonated DOC removal by adsorption and biological

80 degradation over time. (The period A, B, and C represent the physical adsorption, concurrent

81 adsorption/biological degradation and biological degradation processes respectively, and the

82 period D represents the loss of biodegradation efficiency.)

83



84

85 **Fig. S3** Correlations between FRI of each region and (a) DCAN FP, (b) DCAcAm FP

86 **Table S1** Regular water quality parameters in raw water and in the influent and effluent of BAC

Parameters	Sampling time	Before BAC	After BAC
COD_{Mn} (mg/L)	summer	1.43	0.93
	winter	1.66	1.20
DOC （mg/L）	summer	1.96	0.89
	winter	1.59	0.92
DON （mg/L）	summer	0.27	0.20
	winter	0.42	0.35
TDN (mg/L)	summer	0.71	0.64
	winter	0.97	0.86
NO_3^--N (mg/L)	summer	0.37	0.41
	winter	0.44	0.45
NH_4^+-N (mg/L)	summer	0.07	0.03
	winter	0.11	0.06

87

Table S2 The integration of each fluorescence region and their removal rates by BAC

	Ⅰ	Ⅱ	Ⅲ	Ⅳ	Ⅴ
SI[a]	283044	235188	174317	245584	140375
SE[a]	206514	164084	152168	186328	121492
SR[c] (%)	27	29	12	24	13
WI[b]	323435	270121	190090	268321	159778
WE[b]	271235	221465	172057	228756	146595
WR[c] (%)	16	18	9	14	8

[a]: SI and SE respectively refer to the integration of each fluorescence region in the influent and effluent of BAC in summer.

[b]: WI and WE respectively refer to the integration of each fluorescence region in the influent and effluent of BAC in winter.

[c]: SR and WR refer to the removal rates of these regional integral by BAC in summer and winter, respectively..

Table S3 Concentration of amino acids and proteins in raw water and in the influent and effluent of BAC in summer

Amino acids	Full Name	Raw water	Before BAC	After BAC
Asn	Asparagine	N/A[a]	N/A	N/A
Cys	Cysteine	N/A	N/A	N/A
Trp	Tryptophan	N/A	N/A	N/A
Glu	Glutamic acid	2.77	1.24	0.86
Ser	Serine	0.94	0.51	0.37
Gly	Glycine	2.18	0.94	0.61
Asp	Aspartic acid	0.64	0.26	0.18
Ala	Alanine	0.87	0.47	0.35
Lys	Lysine	0.79	0.37	0.36
Thr	Threonine	0.36	0.20	0.16
His	Histidine	0.61	0.28	0.31
Arg	Arginine	0.34	0.13	0.10
Ile	Isoleucine	0.18	0.05	0.04
Leu	Leucine	0.11	0.04	0.03
Phe	Phenylalanine	0.08	0.15	0.16
Pro	Proline	0.15	0.19	0.15
Val	Valine	0.05	0.08	0.05
Gln	Glutamine	0.09	0.02	N/A
Tyr	Tyrosine	N/A	N/A	N/A
Met	Methionine	N/A	N/A	N/A
Total AAs	Total amino acids	10.16	4.94	3.71
Pro	proteins	4.89	2.64	1.28

[a]: N/A refers to that the concentration of the amino acid was under the limit of detection.

The unit of all the amino acid concentrations is μmol/L, the unit of proteins concentration is mg/L.

Table S4 Concentration of amino acids and proteins in raw water and in the influent and effluent of BAC in winter

Amino acids	Full Name	Raw water	Before BAC	After BAC
Asn	Asparagine	N/A[a]	N/A	N/A
Cys	Cysteine	N/A	N/A	N/A
Trp	Tryptophan	N/A	N/A	N/A
Glu	Glutamic acid	3.78	1.65	1.48
Ser	Serine	1.35	0.79	0.67
Gly	Glycine	3.16	1.38	1.23
Asp	Aspartic acid	0.95	0.44	0.39
Ala	Alanine	1.18	0.69	0.58
Lys	Lysine	1.06	0.53	0.57
Thr	Threonine	0.50	0.29	0.25
His	Histidine	0.94	0.48	0.55
Arg	Arginine	0.40	0.18	0.16
Ile	Isoleucine	0.15	0.05	0.06
Leu	Leucine	0.17	0.07	0.06
Phe	Phenylalanine	0.13	0.19	0.22
Pro	Proline	0.21	0.18	0.13
Val	Valine	0.02	0.02	0.02
Gln	Glutamine	0.12	0.03	N/A
Tyr	Tyrosine	0.02	N/A	N/A
Met	Methionine	N/A	N/A	N/A
Total AAs	Total amino acids	14.14	6.97	6.37
Pro	proteins	5.67	3.10	2.03

[a]: N/A refers to that the concentration of the amino acid was under the limit of detection.

The unit of all the amino acid concentrations is μmol/L, the unit of proteins concentration is mg/L.

References

Boon, N., Goris, J., Vos, P.D., Verstraete, W., Top, E.M., 2000. Bioaugmentation of Activated Sludge by an Indigenous 3-Chloroaniline-Degrading Comamonas testosteroni Strain, I2gfp. Appl Environ Microbiol 66, 2906-2913.

Cohen, S.A., Michaud, D.P., 1993. Synthesis of a Fluorescent Derivatizing Reagent, 6-Aminoquinolyl-N -Hydroxysuccinimidyl Carbamate, and Its Application for the Analysis of Hydrolysate Amino Acids via High-Performance Liquid Chromatography. ANAL BIOCHEM 211, 279-287.

Dotson, A., Westerhoff, P., 2009. Occurrence and removal of amino acids during drinking water treatment. Journal 101, 101-115.

Frølund, B., Griebe, T., Nielsen, P.H., 1995. Enzymatic-Activity in the Activated-Sludge Floc Matrix. Applied Microbiology & Biotechnology 43, 755-761.

Lautenschlager, K., Hwang, C., Ling, F., Liu, W.T., Boon, N., Köster, O., Egli, T., Hammes, F., 2014. Abundance and composition of indigenous bacterial communities in a multi-step biofiltration-based drinking water treatment plant. WATER RES 62, 40-52.

Martens, D.A., Loeffelmann, K.L., 2003. Soil amino acid composition quantified by acid hydrolysis and anion chromatography-pulsed amperometry. Journal of Agricultural & Food Chemistry 51, 6521-6529.

Munch, D., Hautman, D., 1995. Method 551.1: Determination of chlorination disinfection byproducts, chlorinated solvents , and halogenated pesticides/ herbicides in drinking water by liquid-liquid extraction and gas chromatography with electron-capture detection.

Telias, A., White, J.R., Pahl, D.M., Ottesen, A.R., Walsh, C.S., 2011. Bacterial community diversity and variation in spray water sources and the tomato fruit surface. BMC Microbiology,11,1(2011-04-21) 11, 81.

Yu, S., Lin, T., Chen, W., Tao, H., 2015. The toxicity of a new disinfection by-product, 2,2-dichloroacetamide (DCAcAm), on adult zebrafish (Danio rerio) and its occurrence in the chlorinated drinking water. CHEMOSPHERE 139, 40-46.