Supplementary Information

for

Formation and toxicity alteration of brominated halonitromethanes from the intracellular organic matter of *Chlorella vulgaris* during UV/chlorine disinfection

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Text S1. Method for detecting the concentration of Br-HNMs

Before preparing the standard BNM, DBNM, TBNM, BCNM, BDCNM, and DBCNM, the standard stock solutions of the six substances must first be prepared. Accurately weigh a certain amount of each of the six substances using a micro-sampler, and add them separately to 500 mL amber volumetric flasks. Then, add methyl tert-butyl ether to the mark, shake well, and transfer to 500 mL amber reagent bottles. This will serve as the standard stock solution. Next, use a pipette to take the required volumes of the six substances and prepare standard samples with concentrations of 5 μg/L, 10 μg/L, 20 μg/L, 50 μg/L, 100 μg/L, 300 μg/L, and 500 μg/L, using methyl tert-butyl ether as the solvent. Add 1 mL of each concentration sample to a 2 mL amber injection vial. Then, analyse these samples using gas chromatography to obtain the standard calibration curves for the six substances.

Sample analysis was performed using an Agilent HP6890 gas chromatograph and an HP-1 chromatographic column (30 m × 0.32 mm × 0.25 μm). The injection port temperature was set at 235°C, the detector temperature at 280°C, and nitrogen gas was used as the carrier gas at a flow rate of 1.0 mL/min. The initial temperature was 50°C, which was held for 5 min, followed by an increase to 140°C at a rate of 10°C/min, and then raised to 280°C at a rate of 20°C/min.

The standard curve equations for each Br-HNMs were obtained through

experimental measurements and regression, as follows:

The standard curve equation for BNM: Area = $492.72C_{BNM}$ - 4951.76, R^2 = 0.9971;

The standard curve equation for DBNM: Area = 468.73_{CDBNM} - 2878.94, R^2 = 0.9996;

The standard curve equation for TBNM: Area = $161.42C_{TBNM}$ - 1031.86, R^2 = 0.9995;

The standard curve equation for BCNM: Area = $421.24C_{BCNM}$ + 7365.63, R^2 = 0.9988;

The standard curve equation for BDCNM: Area = $258.57C_{BDCNM}$ - 3487.97, R^2 = 0.9973;

The standard curve equation for DBCNM: Area = $200.72C_{TCNM}$ - 1828.64, R^2 = 0.9991.

The above curves show good linearity, with correlation coefficients greater than 0.997, meeting the requirements for quantitative analysis. These provide a reliable basis for quantitatively determining Br-HNMs in real samples. The detection limits for BNM, DBNM, TBNM, BCNM, BDCNM, and DBCNM were 0.12, 0.03, 3.59, 0.05, 2.08, and 2.33 μg/L, respectively.

Text S2. Culture Medium Formula and Cultivation Method for *Chlorella vulgaris*

The composition of the culture medium for *Chlorella vulgaris* is as follows: 200 mg/L $(NH_4)_2SO_4$, 30 mg/L [$Ca(H_2PO_4)_2 \cdot 2H_2O$ + $CaSO_4 \cdot H_2O$], 80 mg/L $MgSO_4 \cdot 7H_2O$, 100 mg/L $NaHCO_3$, 25 mg/L KCl, 0.150 mL/L $FeCl_3$ (1%), 2.86 mg/L H_3BO_3, 1.81 mg/L $MnCl_2 \cdot 4H_2O$, 0.222 mg/L $ZnSO_4 \cdot 7H_2O$, 0.0177 mg/L MoO_3 (85%), 0.079 mg/L $CuSO_4 \cdot 5H_2O$, and 0.5 mL/L soil leachate. The pH of the medium is adjusted to 7.0-7.2 using 0.1 M Na_2CO_3.

Soil Leachate Preparation Method: Take 0.1 kg of unfertilized garden soil and place it in a beaker or flask. Add 200 mL of distilled water, seal the bottle with a breathable stopper, and heat in boiling water for 2 hours. After cooling for several hours, filter the solution under sterile conditions. Collect the supernatant, and add sterilised distilled water to make the total volume 200 mL. The soil leachate is stored at 4°C for later use.

First Inoculation of *Chlorella vulgaris*: Dilute the algal inoculum with distilled water and add it to a 50 mL culture bottle. Place the bottle in a light incubator at a constant temperature of (25 ± 1)°C under weak light conditions for 2-3 days. Once the algae are activated, proceed with further culturing. When culturing *Chlorella vulgaris*, prepare the culture medium according to the formula mentioned above. Transfer 250 mL of the prepared medium into a 500 mL conical flask, sterilize it by autoclaving at 123.0 KPa for 20 min. After cooling, inoculate the medium with *Chlorella vulgaris* under sterile conditions, seal the flask with kraft paper and cotton thread, and place it

in a constant temperature light incubator for cultivation. The light cultivation temperature is (25 ± 1)°C.

Text S3. Method for determining the concentration of *Chlorella vulgaris*

The concentration of *Chlorella vulgaris* was determined by spectrophotometry. The transmittance trends for different concentrations of algae solution were consistent, and it was found that the peak absorbance for *Chlorella vulgaris* occurred at 680 nm. The concentration of algal solution was calculated by Equation (S1):

$$C=\frac{A}{KL}=-\frac{\ln(T)}{L\pi R^2} \quad , \quad (S1)$$

where C is the concentration of *Chlorella vulgaris* (10^8 cells/L), A is absorbance, K is the absorption coefficient of substance, T is transmittance, L is cuvette thickness (take 1cm), R is the average radius of *Chlorella vulgaris* (μm).

Text S4. Determination of chlorine and nitrogen compounds

Determination of Free Chlorine and Total Chlorine

The concentrations of free chlorine and total chlorine are determined using the DPD-FAS method by titration, following the procedure outlined in HJ 585-2010. The detection limit for this standard (expressed as Cl_2) is 0.02 mg/L, and the measurement range (expressed as Cl_2) is from 0.08 mg/L to 5 mg/L. Therefore, for free chlorine and total chlorine concentrations exceeding the upper measurement limit, dilution can be performed before measurement. This standard defines free chlorine as the chlorine present in the form of hypochlorous acid, hypochlorite ions, and dissolved chlorine gas; combined chlorine as chlorine present in the form of chloramines and organic chloramines; and total chlorine as either free chlorine or combined chlorine, or a mixture of both.

The principle for determining free chlorine is: under pH conditions of 6.2-6.5, free chlorine reacts with N, N-diethyl-1,4-phenylenediamine (DPD) to form a red compound, which is titrated with ammonium iron(II) sulfate standard solution until the red colour disappears.

The principle for determining total chlorine is: under pH conditions of 6.2-6.5, in the presence of excess potassium iodide, total chlorine (elemental chlorine, hypochlorous acid, hypochlorite ions, and chloramines) reacts with DPD to form a red compound, which is titrated with ammonium iron(II) sulfate standard solution until

the red color disappears.

Free Chlorine Measurement Method: In a 250 mL conical flask, add 15.0 mL phosphate buffer solution, 5.0 mL DPD solution, and the sample. Mix well. Immediately titrate with ammonium iron(II) sulfate standard solution until the solution becomes colorless. Record the volume of titrant consumed in milliliters.

Total Chlorine Measurement Method: In a 250 mL conical flask, add 15.0 mL phosphate buffer solution, 5.0 mL DPD solution, and the sample. Add 1g potassium iodide and mix well. After 2 min, titrate with ammonium iron(II) sulfate standard solution until the solution becomes colorless. If pink color reappears within 2 min, continue titrating until the solution is colorless, and record the volume of titrant consumed in milliliters.

Determination of Total Nitrogen

Total nitrogen is determined using the alkaline persulfate digestion ultraviolet spectrophotometric method, following the procedure in HJ 636-2012. The detection limit for this standard, with a sample volume of 10 mL, is 0.05 mg/L, and the measurement range is from 0.20 mg/L to 7.00 mg/L. Total nitrogen in this standard refers to the total nitrogen present in dissolved and suspended forms in the sample, including nitrite nitrogen, nitrate nitrogen, inorganic ammonium salts, dissolved ammonia, and most nitrogen compounds in organic matter.

The principle for determining total nitrogen is: under a temperature of 120-124°C,

the alkaline persulfate potassium solution converts the nitrogen in nitrogen-containing organic matter into nitrate. The absorbance at wavelengths of 220 nm and 275 nm is measured using ultraviolet spectrophotometry. The corrected absorbance ($A = A_{220} - 2A_{275}$) is proportional to the total nitrogen (as N).

Total Nitrogen Measurement Method: Take 5 mL of the sample to be tested and add it to a 25 mL colorimetric tube. Add 5 mL of alkaline persulfate potassium solution. Seal the tube with a stopper and secure it with gauze and string. Heat the tube in a high-pressure steam boiler at 120°C-124°C for 30 min. After cooling, add 1 mL of (1+9) hydrochloric acid, and dilute with water to the 25 mL mark. Measure the absorbance in a 10 mm quartz colorimetric cell using an ultraviolet spectrophotometer at 220 nm and 275 nm, and calculate the total nitrogen concentration based on the sample volume and the standard curve.

Determination of Ammonia Nitrogen

Ammonia nitrogen is determined using the Nessler reagent spectrophotometric method, following the procedure in HJ 535-2009. The detection limit for this standard, with a sample volume of 50 mL and using a 20 mm colorimetric cell, is 0.025 mg/L, and the measurement range (expressed as N) is from 0.10 mg/L to 2.0 mg/L.

The principle for determining ammonia nitrogen is: ammonia nitrogen, present in the form of free ammonia or ammonium ions, reacts with Nessler reagent to form a

light reddish-brown complex, and the absorbance of the complex is proportional to the ammonia nitrogen content. Absorbance is measured at a wavelength of 420 nm.

Ammonia Nitrogen Measurement Method: Take 10 mL of the sample to be tested and add it to a 50 mL colorimetric tube. Add 1.0 mL of potassium sodium tartrate solution, mix well, then add 1.0 mL of Nessler reagent and mix again. If residual chlorine is present in the sample, add sodium thiosulfate solution to remove the residual chlorine. After standing for 10 min, measure the absorbance at 420 nm using a 10 mm colorimetric cell, and calculate the ammonia nitrogen concentration based on the sample volume and the standard curve.

Determination of Nitrate Nitrogen

Nitrate nitrogen is determined using the ultraviolet spectrophotometric method, following the procedure in HJ/T 346-2007. The minimum detection concentration for this standard is 0.08 mg/L, with a measurement range from 0.32 mg/L to 4 mg/L.

The principle for determining nitrate nitrogen is: nitrate ions absorb light at a wavelength of 220 nm, and the absorbance is used to quantify nitrate nitrogen. Organic matter dissolved in water also absorbs at 220 nm, but nitrate ions do not absorb at 275 nm. Therefore, a second measurement is made at 275 nm to correct the nitrate nitrogen value (A_ corrected = A220 - 2A275).

Nitrate Nitrogen Measurement Method: Take 10 mL of the sample to be tested and add it to a 50 mL colorimetric tube. Add 1.0 mL of 1 mol/L hydrochloric acid solution

and 0.1 mL of amino sulfonic acid solution. Shake well, and then measure the absorbance at wavelengths of 220 nm and 275 nm using a 10 mm quartz colourimetric cell. Calculate the nitrate nitrogen concentration based on the sample volume and the standard curve.

Table

Table S1. Chain reaction of active species

No.	Reaction equation	Rate constant
1	$HOCl + Br^- \rightarrow HOBr + Cl^-$	$6.84 \times 10^3\ M^{-1}\ s^{-1}$
2	$HOBr \rightarrow H^+ + OBr^-$	$7.90 \times 10^1\ s^{-1}$
3	$HOBr/OBr^- + hv \rightarrow Br^\bullet + HO^\bullet/O^{\bullet-}$	-
4	$HOBr + HO^\bullet \rightarrow BrO^\bullet + H_2O$	$2.00 \times 10^9\ M^{-1}\ s^{-1}$
5	$OBr^- + HO^\bullet \rightarrow BrO^\bullet + OH^-$	$4.20 \times 10^9\ M^{-1}\ s^{-1}$
6	$HO^\bullet + Br^- \rightarrow BrOH^{\bullet-}$	$1.30 \times 10^{10}\ M^{-1}\ s^{-1}$
7	$BrOH^{\bullet-} + Br^- \rightarrow Br_2^{\bullet-} + OH^-$	$1.90 \times 10^8\ M^{-1}\ s^{-1}$
8	$BrOH^{\bullet-} + H^+ \rightarrow Br^\bullet + H_2O$	$4.40 \times 10^{10}\ M^{-1}\ s^{-1}$
9	$Br^\bullet + Br^- \rightarrow Br_2^{\bullet-}$	$1.20 \times 10^{10}\ M^{-1}\ s^{-1}$
10	$Br^\bullet + Br^\bullet \rightarrow Br_2$	$1.00 \times 10^9\ M^{-1}\ s^{-1}$
11	$Br_2 + Br^- \rightarrow Br_3^-$	$9.60 \times 10^8\ M^{-1}\ s^{-1}$
12	$Cl^\bullet + Br^- \rightarrow BrCl^{\bullet-}$	$1.20 \times 10^{10}\ M^{-1}\ s^{-1}$
13	$Cl_2^{\bullet-} + Br^- \rightarrow BrCl^{\bullet-} + Cl^-$	$4.00 \times 10^9\ M^{-1}\ s^{-1}$
14	$ClOH^{\bullet-} + Br^- \rightarrow OH^- + BrCl^{\bullet-}$	$1.00 \times 10^9\ M^{-1}\ s^{-1}$
15	$BrCl^{\bullet-} + Br^- \rightarrow Br_2^{\bullet-} + Cl^-$	$8.00 \times 10^9\ M^{-1}\ s^{-1}$

Table S2. List of experimental instruments

Instrument Name	Model / Specification	Manufacturer
Gas Chromatograph	HP6890	Agilent Technologies Co., Ltd.
Chromatographic Column	HP-1 (30 m×0.32 mm×0.25μm)	Agilent Technologies Co., Ltd.
Magnetic Stirrer	78-1	Changzhou Guohua Electric Appliance Co., Ltd.
Illuminated Incubator	MGC-300A	Shanghai Yiheng Scientific Instrument Co., Ltd.
UV-Vis Spectrophotometer	Model 754	Shanghai Jinghua Technology Instrument Co., Ltd.
Centrifuge	L600	Xiangyi Centrifuge Instrument Co., Ltd.
Electronic Balance	AR124CN	Ohaus Instruments Co., Ltd.
UV Radiometer	UV-B	Photoelectric Instrument Factory of Beijing Normal University
pH Meter	PHS-3C	Shanghai Leici Instrument Factory
Fiber Optic Spectrometer	USB4000	Ocean Optics
Variable Speed Multi-purpose Shaker	HY-6	Changzhou Guohua Electric Appliance Co., Ltd.

Table S3. List of chemical reagents

Reagent Name	Specification / Purity	Manufacturer
Bromonitromethane (97%)	200 mg	Quality Control Chemicals
Dibromonitromethane (97%)	200 mg	Quality Control Chemicals
Tribromonitromethane (100%)	100 mg	Quality Control Chemicals
Bromochloronitromethane (97%)	100 mg	Quality Control Chemicals
Bromodichloronitromethane (97%)	100 mg	Quality Control Chemicals
Dibromochloronitromethane (97.4%)	100 mg	Quality Control Chemicals
Chlorella strain	13 mL	Institute of Hydrobiology, Chinese Academy of Sciences
Methyl tert-butyl ether (MTBE)	500 mL	Sinopharm Chemical Reagent Co., Ltd.
Disodium hydrogen phosphate	500 g	Sinopharm Chemical Reagent Co., Ltd.
Sodium dihydrogen phosphate	500 g	Sinopharm Chemical Reagent Co., Ltd.
Sodium hypochlorite	500 mL	Tianjin Kemiou Chemical Reagent Co., Ltd.
Concentrated hydrochloric acid	500 mL	Sinopharm Chemical Reagent Co., Ltd.
Potassium sodium tartrate	500 g	Chengdu Kelong Chemical Reagent Co., Ltd.
Potassium persulfate	500 g	Sigma-Aldrich (Sigma Reagent)
Sodium hydroxide	500 g	Sinopharm Chemical Reagent Co., Ltd.

Continued Table S3. List of chemical reagents

Reagent Name	Specification / Purity	Manufacturer
Sodium sulfamate	25 g	Shanghai Yuanye Bio-Technology Co., Ltd.
Nessler's reagent (HgI2-KI-NaOH)	500 mL	Aladdin Biochemical Technology Co., Ltd.
N,N-Diethyl-1,4-phenylenediamine (DPD)	1 g	Shanghai Jiuding Chemical Technology Co., Ltd.
Ammonium ferrous sulfate hexahydrate (FAS)	500 g	Sinopharm Chemical Reagent Co., Ltd.
Potassium iodide	25 g	Shanghai Macklin Biochemical Co., Ltd.
Concentrated sulfuric acid	500 mL	Sinopharm Chemical Reagent Co., Ltd.
Orthophosphoric acid	500 mL	Shanghai Titan Scientific Co., Ltd.
Potassium dichromate	50 g	Sinopharm Chemical Reagent Co., Ltd.
Barium diphenylamine sulfonate	25 g	Sinopharm Chemical Reagent Co., Ltd.
Nessler's reagent	500 mL	Aladdin Biochemical Technology Co., Ltd.
Sodium thiosulfate	500 g	Sinopharm Chemical Reagent Co., Ltd.
Ammonium chloride	500 g	Sinopharm Chemical Reagent Co., Ltd.
Potassium nitrate	500 g	Sinopharm Chemical Reagent Co., Ltd.
tert-Butanol	500 mL	Sinopharm Chemical Reagent Co., Ltd.

Table S4. Physicochemical properties of Br-HNMs

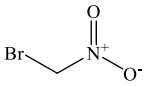
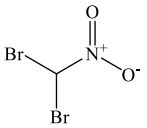
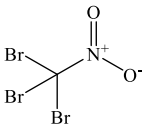
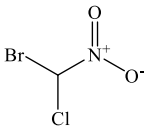
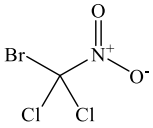
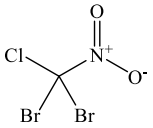
Compounds	structural formula	molecular formula	molecular weight	boiling point (℃, 760mmHg)	Solubility (mg/L,20℃)
BNM		CH_2BrNO_2	139.94	147.47	18680
DBNM		$CHBr_2NO_2$	218.83	152.7	4577
TBNM		CBr_3NO_2	297.73	155.9	227.5
BCNM		$CHClBrNO_2$	174.38	132.7	9163
BDCNM		CCl_2BrNO_2	208.83	115.5	1007
DBCNM		$CClBr_2NO_2$	253.28	134.9	486.1

Table S5. The water quality indicators of STP and WSP

Actual water samples	pH	CODcr (mg/L)	DOC (mg/L)	TN (mg/L)	NH_3-N (mg/L)	TP (mg/L)
STP	6.96	13.20	6.03	20	0.05	0.12
WSP	7.93	2.48	3.48	1.8	0.035	7.4

* The real water samples were collected on August 22, 2019 (summer).

Table S6. The LC_{50} and 50% Tail DNA of six Br-HNMs

Br-HNMs	LC_{50} (M)	50%TDNA (M)
BNM	7.08×10^{-6}	1.36×10^{-4}
DBNM	6.09×10^{-6}	2.62×10^{-5}
TBNM	8.57×10^{-6}	6.99×10^{-5}
BCNM	4.05×10^{-5}	1.65×10^{-4}
BDCNM	1.32×10^{-5}	6.32×10^{-5}
DBCNM	6.88×10^{-6}	1.43×10^{-4}

Figure

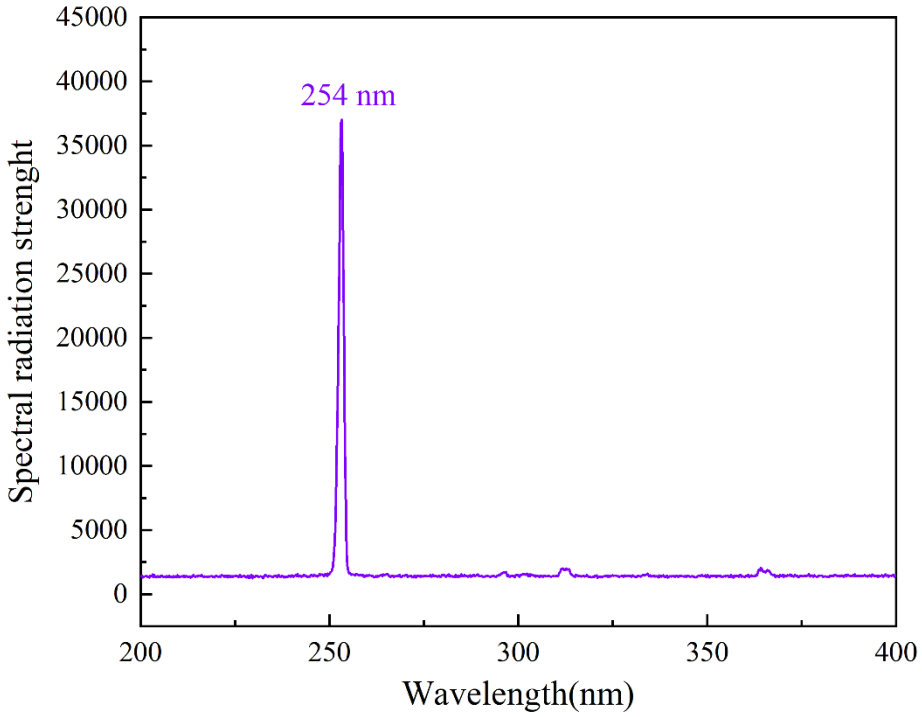


Fig. S1 The emission spectrum of the UV lamp

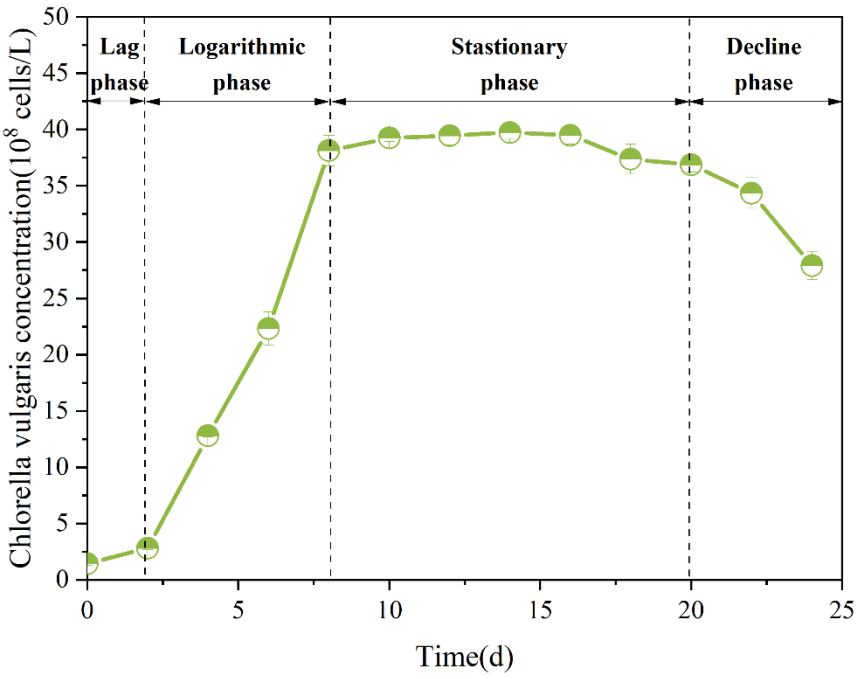


Fig. S2 Growth curve of *Chlorella vulgaris*

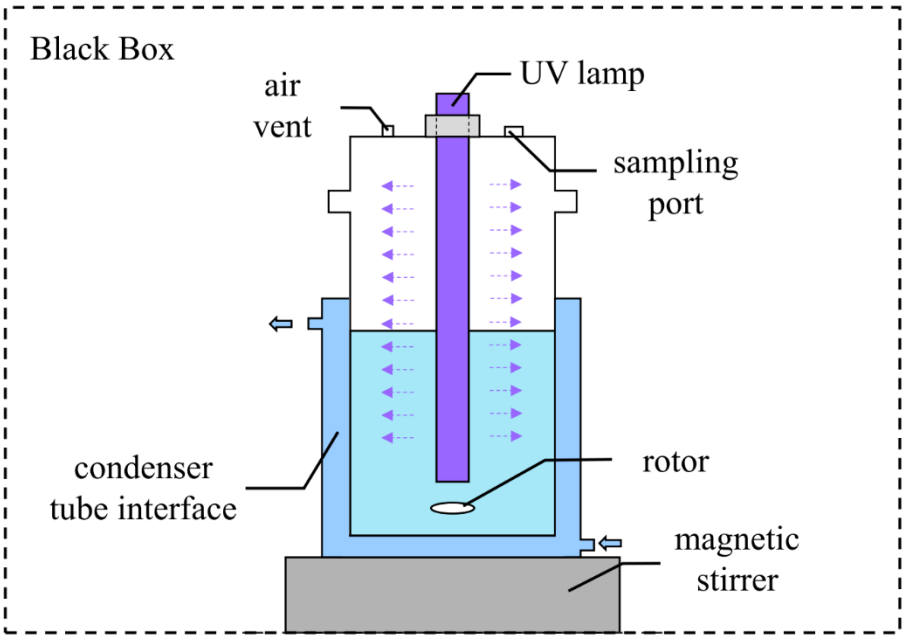


Fig. S3 Schematic diagram of quartz photochemical reactor (length: 20.0 cm, diameter: 9.0 cm, thickness: 1.0 cm)

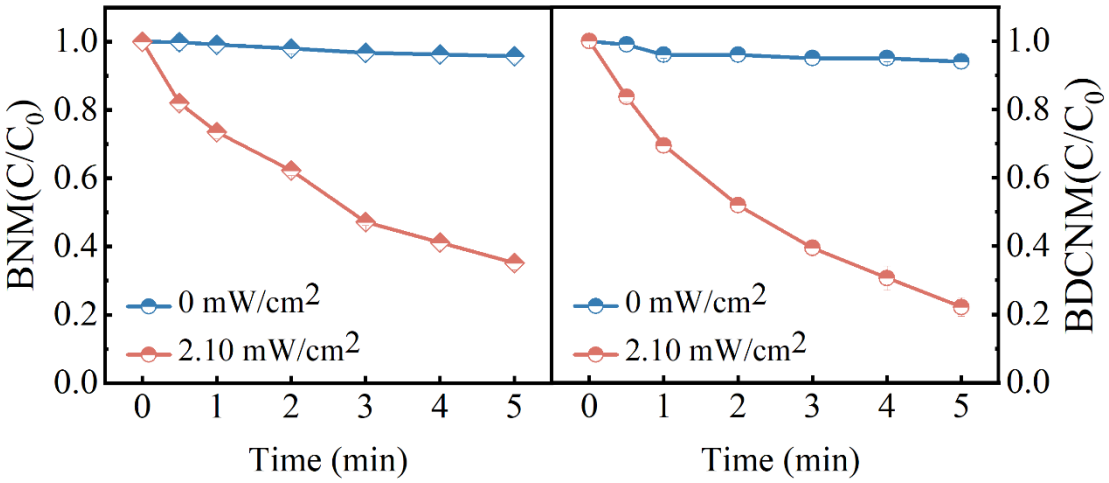


Fig. S4 The effect of UV on the degradation of BNM and BDCNM.

Experimental conditions: [BNM]= 200.0 μg/L, [DBCNM]= 200.0 μg/L, pH=7.0, [UV intensity]=0, 2.10 mW/cm^2, [Br^-]=0, 0.5, 1 mg/L

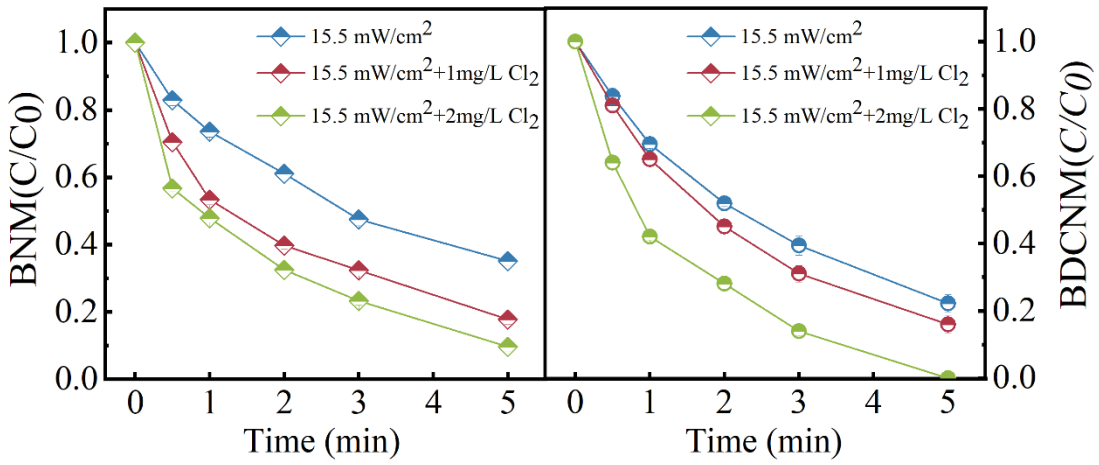


Fig. S5. The effect of different free chlorine concentrations on the degradation of BNM and BDCNM.

Experimental conditions: [BNM]= 200.0 μg/L, [DBCNM]= 200.0 μg/L, pH=7.0, [UV intensity]= 2.10 mW/cm^2, [Cl^-]=0, 1, 2 mg/L

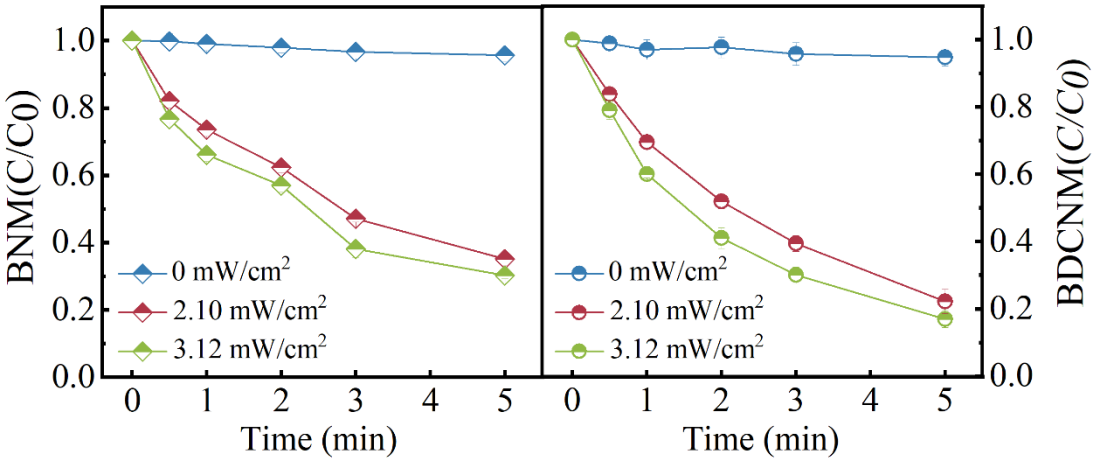


Fig. S6. The effect of different UV intensities on the degradation of BNM and BDCNM.

Experimental conditions: [BNM]= 200.0 μg/L, [DBCNM]= 200.0 μg/L, pH=7.0, [UV intensity]= 0, 2.10, 3.12mW/cm^2