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CONTENTS

Aquatic environment	
Occurrence of selected aliphatic amines in source water of major cities in China	
Haifeng Zhang, Shuoyi Ren, Jianwei Yu, Min Yang · · · · · 18	85
Selection of magnetic anion exchange resins for the removal of dissolved organic and inorganic matters	
Qiongjie Wang, Aimin Li, Jinnan Wang, Chengdong Shuang · · · · · 18	91
Reductive transformation and detoxification mechanism of 2,4-dinitrochlorobenzene in combined zero valent iron	
and anaerobic-aerobic process	
Jinyou Shen, Zongyuan Zhou, Changjin Ou, Xiuyun Sun, Jiansheng Li, Weiqing Han, Lin Zhou, Lianjun Wang19	00
Fractionation of residual Al in natural water treatment from reservoir with poly-aluminum-silicate-chloride (PASiC):	
Effect of OH/Al, Si/Al molar ratios and initial pH	
Zhonglian Yang, Baoyu Gao, Yan Wang, Yaqin Zhao, Qinyan Yue·····19	08
Effect of solid contents on the controlled shear stress rheological properties of different types of sludge	
Ting Li, Yili Wang, Yujing Dong · · · · 19	17
Optimizing the operation of the Qingshitan Reservoir in the Lijiang River for multiple human interests and	
quasi-natural flow maintenance	
Qiuwen Chen, Duan Chen, Ruiguang Han, Ruonan Li, Jinfeng Ma, Koen Blanckaert · · · · · 19	23
Zeolite (Na) modified by nano-Fe particles adsorbing phosphate in rainwater runoff	
Lili Gan, Jiane Zuo, Bangmi Xie, Peng Li, Xia Huang · · · · · 19	29
Contamination by persistent toxic substances in surface sediment of urban rivers in Chaohu City, China	
Feipeng Li, Haiping Zhang, Xiangzhou Meng, Ling Chen, Daqiang Yin19	34
Fatty acids and algal lipids as precursors of chlorination by-products	
Yan Liang, Yuen Shan Lui, Huachang Hong · · · · 19	42
Atmospheric environment	
Uptake of isoprene, methacrylic acid and methyl methacrylate into aqueous solutions of sulfuric acid and hydrogen peroxide	
Ze Liu, Maofa Ge, Weigang Wang · · · · 19	47
Comparison of PM ₁₀ concentrations and metal content in three different sites of the Venice Lagoon:	
An analysis of possible aerosol sources	
Daniele Contini, Franco Belosi, Andrea Gambaro, Daniela Cesari, Angela Maria Stortini, Maria Chiara Bove	54
Seasonal trend of ambient PCDD/Fs in Tianjin City, northern China using active sampling strategy	
Lei Ding, Yingming Li, Pu Wang, Xiaomin Li, Zongshan Zhao, Qinghua Zhang, Ting Tuan, Guibin Jiang19	66
Ultrafine particle emission characteristics of diesel engine by on-board and test bench measurement	
Cheng Huang, Diming Lou, Zhiyuan Hu, Piqiang Tan, Di Yao, Wei Hu, Peng Li, Jin Ren, Changhong Chen19	72
N-doped mesoporous alumina for adsorption of carbon dioxide	
Jayshri A. Thote, Ravikrishna V. Chatti, Kartik S. Iyer, Vivek Kumar, Arti N. Valechha, Nitin K. Labhsetwar,	
Rajesh B. Biniwale, M. K. N. Yenkie, Sadhana S. Rayalu·····19	79
Terrestrial environment	
Extraction of heavy metals from e-waste contaminated soils using EDDS	
Renxiu Yang, Chunling Luo, Gan Zhang, Xiangdong Li, Zhenguo Shen·····19	85
Characterization of contamination, source and degradation of petroleum between upland and paddy fields based	
on geochemical characteristics and phospholipid fatty acids	
Juan Zhang, Renqing Wang, Xiaoming Du, Fasheng Li, Jiulan Dai · · · · · · 19	95
Environmental biology	
Bacterial diversity and distribution in the southeast edge of the Tengger Desert and their correlation with soil enzyme activities	
Wei Zhang, Gaosen Zhang, Guangxiu Liu, Zhibao Dong, Tuo Chen, Manxiao Zhang, Paul J. Dyson, Lizhe An20	04
Biodegradation of <i>p</i> -cresol by aerobic granules in sequencing batch reactor	
Farrukh Basheer, I. H. Farooqi · · · · · · · 20	12
Environmental health and toxicology	
Characterisation of acute toxicity, genotoxicity and oxidative stress posed by textile effluent on zebrafish	
Wenjuan Zhang, Wei Liu, Jing Zhang, Huimin Zhao, Yaobin Zhang, Xie Quan, Yihe Jin20	19
Characterization of cytotoxicity of airborne particulates from urban areas of Lahore	
Badar Ghauri, M. Mansha, Christian Khalil	28
Serial parameter: CN 11.2620/Y*1080*m*150*en*D*2012.11	300





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Fatty acids and algal lipids as precursors of chlorination by-products

Yan Liang^{1,3,*}, Yuen Shan Lui¹, Huachang Hong^{2,*}

Croucher Institute for Environmental Sciences (CIES) and Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong, China
 College of Geography and Environmental Sciences, Zhejiang Normal University, Jinhua 321004, China
 Centre for Food Safety and Environmental Technology, Guangzhou Institute of Advanced Technology, Chinese Academy of Sciences,
Guangzhou 511458, China

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Abstract

Six common algal fatty acids (FAs) with different numbers of double bonds, lipophilic fractions and proteins extracted from the diatom *Navicula pelliculosa* and algal cells were chlorinated to evaluate their potential in generating disinfection by-products (DBPs). The result showed that the more double bonds in the FAs, the higher the amounts of chloroform and dichloroacetic acid (DCAA) produced, but such a pattern was not observed for trichloroacetic acid (TCAA). Based on the previously reported composition of fatty acids in algal lipids, the DBP generation potentials of algal lipids were calculated. These predicted values were much lower than those measured in the chlorinated algal lipophilic fraction, suggesting unknown lipophilic fraction(s) served as potent DBPs precursors. Another calculation attempted to predict DBP production in algal cells based on algal lipid and protein composition, given quantified measured DBP production per unit algal lipid and proteins. The analysis showed that the observed DBP production was similar to that predicted (< 35% difference), suggesting that algal biochemical compositions may serve as a bioindicator for preliminary estimation of chloroform, DCAA and TCAA formation upon chlorinating algae.

Key words: algal lipid; algal-derived organic materials; chlorination by-products; fatty acids

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Introduction

Chlorination has been applied in drinking water treatment as a major chemical disinfection measure throughout the world, including in Hong Kong and mainland China. Disinfection by-products (DBPs) are generated during chlorination. In Hong Kong, our 3-year monitoring data revealed that in the drinking water reservoirs one major source of the organic precursors of DBPs was algal-derived organic matter, with a specific UV₂₅₄ value (SUV₂₅₄) of 1.78 L/(mg·m) (Hong et al., 2008b). This may be due to contamination by agricultural activity, discharges of domestic wastewater and industrial effluents in the source water (Liang et al., 2008), resulting in eutrophication and algal growth/blooms.

Algal cellular biochemical composition varies with factors such as algal species, growth phases, nutrient availability, and other environmental factors (Lynn et al., 2000; Woods et al., 2003; Hong et al., 2008a; Das et al., 2011). For example, an increase in lipid classes, especially triglycerides, was observed in many freshwater algae (including cyanobacteria) under nutrient-limiting conditions (Ahlgren et al., 1992; Lynn et al., 2000). Even with similar nutritional supply, algae grown under low levels of

light intensity and temperature, such as in places of high latitude, tend to accumulate high levels of proteins and lipids to maintain photosynthesis and protect cells against freezing (Smith and Morris, 1980; Denicola, 1996; Woods et al., 2003). Additionally, carbohydrates and lipids also increased when a natural algal bloom peaked (Kreeger et al., 1997).

However, information on lipids as precursors of DBPs upon chlorination is rare. Only one available reference reported that DBP formation from chlorination of lipids with high levels of double bonds was extensive (Fukayama et al., 1986). As algal growth in Hong Kong's source water was phosphorus-limited (Hong et al., 2008b), it is likely that this may lead to lipid accumulation in algae, which may cause high DBP production following chlorination.

In this study, the pattern of DBP formation upon chlorination of algal lipid, common algal fatty acids (FAs), algal proteins and cells were analyzed. A dominant genus of diatom, *Navicula pelliculosa*, commonly found in the local drinking water reservoirs (Liang et al., 2008), was selected for the experiments. The objectives of this study were: (1) to determine the extent of DBP formation potential of algal lipid, as compared to other algal-derived organic matters (cell, proteins); (2) to examine the link between the degree of saturation of algal lipid/FA and DBP formation potential; (3) to identify whether the percentage of major

^{*} Corresponding authors. E-mail: yliang@hkbu.edu.hk (Yan Liang); honghc@zjnu.cn (Huachang Hong)

biochemical components (lipid and protein) in algae can serve as a predictor of DBP yield upon chlorinating algae.

1 Materials and methods

Navicula pelliculosa (purchased from the University of Texas Culture Collection, USA) was cultured in the DG medium in 5 L glass jars, at $(25 \pm 2)^{\circ}$ C under 1000 lux of illumination with a 14 hr:10 hr light:dark cycle. Daily counting by a light microscope was conducted to monitor the algal growth, and optical density (OD 680 nm) was used to quantify the algal biomass. When the algal growth reached the log phase, the algal biomass was harvested for the subsequent experiments by centrifugation (3000 r/min, 20 min) and rinsed by 0.85% NaCl. All materials and media were sterilized by autoclaving before assembly and operation. Algal proteins were extracted using a plant total protein extraction kit (Sigma, PE0230). The detailed procedure was described in our previous report (Lui et al., 2011). Fatty acids with variable double bonds, including myristic acid (M3128), palmitic acid (P0500), palmitoleic acid (P9417), oleic acid (O1008), linoleic acid (L1376) and linolenic acid (L2376), were purchased from Sigma-Aldrich. The organic carbon content in algal cells, proteins and fatty acids was determined using a TOC analyzer (Shimadzu TOC 5000), after suspending/dissolving these materials in Milli-Q water. The detailed TOC analysis procedure was described in our previous report (Lui et al., 2011).

For algal lipid extraction, freeze-dried N. pelliculosa cells were first ground in a mortar and pestle with 18 mL of hexane:isopropanol (HIP) for 2 min. The solution was then transferred into a Teflon centrifuge tube and placed into a water bath and incubated at 60°C with mixing for 15 min. The centrifuge tube was then centrifuged for 5 min at $1000 \times g$ in order to separate cell debris from the extraction solvent. The supernatant was then transferred to a 90 mL glass bottle with stopper. An additional 18 mL of HIP was added to re-suspend the pellet in the centrifuge tube and washed twice. Ten milliliters of 6.6% sodium sulfate solution was then added into the 90 mL bottle to remove non-lipophilic compounds in the extract and the bottle was shaken for 1 min. All the supernatant in the 90 mL bottle was transferred to a weighed round bottom flask which was fit into a rotary evaporator for the removal of the solvent under vacuum. When all the solvent was evaporated, the round bottom flask was weighed again to obtain the net weight of the lipophilic fraction extracted. The carbon content in the lipids (%) was determined by a CHNS/O analyzer (2400 Series II, Perkin Elmer).

Chlorination was conducted by adding sodium hypochlorite solution (NaClO, Sigma 13440, 49.7 mg Cl₂/mL), with free chlorine determined by titration using N,N-diethyl-*p*-phenylenediamine (DPD) (APHA, 1998). All the dark glass bottles with glass stoppers (80 mL) were washed with detergent, pre-soaked in 10% sulfuric acid for 24 hr and rinsed with Milli-Q water before use. The chlorination condition was at pH 7, 25°C. Solutions containing algal cells, proteins, lipids and fatty acids were

dosed with a Cl₂/DOC ratio of 20 mg Cl₂/mg C (with starting DOC of each sample 50 mg C/L), in order to keep a residue Cl₂ \geqslant 1 mg/L so that the reactions were not chlorine limited. The solutions were stored headspace-free for 0.5, 1, 2, 5, 10, 30, 60 and 120 min at 20°C in the dark. For each reaction interval, samples of the chlorinated solutions were analyzed for DBPs. For each precursor type, chlorination was conducted 3 times.

DBP analyses, including chloroform, dichloroacetic acid (DCAA) and trichloroacetic acid (TCAA), were described in our previous report (Lui et al., 2011). Briefly, CHCl₃ was extracted with pentane and determined according to the standard method (APHA, 1998). DCAA and TCAA were extracted using methyl tertbutyl ether (MTBE), methylated by acidic methanol. 1,2-Dibromopropane was used as the internal standard. The DBPs were determined by a GC-ECD system (with a 30 m \times 0.25 mm \times 0.25 μm HP-5 capillary column) using nitrogen as the carrier gas. The recovery rates for CHCl₃, DCAA and TCAA were 97.5% \pm 6.3%, 101.0% \pm 8.4% and 97.3% \pm 5.3% respectively. The detection limits for these compounds were 9.38, 10.5 and 8.94 nmol/L, respectively.

2 Results and discussion

The result supported the hypothesis that fatty acids served as precursors of DBPs (Fig. 1). After 120 hr chlorination, FAs formed high levels of chloroform (96.6–766 µmol/mol C), DCAA (48.5-187 µmol/mol C) and TCAA (344-640 umol/mol C). Additionally, the yield of chloroform and DCAA was closely related to the degree of saturation of the FAs (Fig. 1), indicating that the more double bonds in the FAs, the higher formation potentials of the DBPs were. For example, linolenic acid (with 3 double bonds per molecule) generally showed higher DBP formation potentials (within 30 sec-120 hr, chloroform 50.7-766 µmol/mol C; DCAA 161-187 µmol/mol C) than myristic acid or palmitic acid (without any double bonds per molecule) (within 30 sec-120 hr, chloroform 21.2-188 µmol/mol C; DCAA 29.9–118 µmol/mol C). This is as expected; double bonds generally served as reactive sites where chlorine or hydroxyl groups were added to the FA molecules and eventually led to the formation of DBPs (Fukayama et al., 1986; Mutton, 1959). Regression analyses further quantified the relationships (Fig. 2). This showed that more than 80% of the variations in the DBP formation potential were explained by the number of double bonds in FAs, and the linear relationships were significant (p < 0.05). No correlation was found between saturation in lipids and TCAA's generation, possibly due to limited sample numbers. More research should be conducted in the future to investigate TCAA generation from lipids under variable chlorination conditions (e.g., pH, temperature, time) with more sample numbers to understand more about the related mechanisms.

The lipid of *N. pelliculosa* was mainly composed of 31% palmitoleic acid (16:1), 26% of eicosapentaenoic acid (20:5), 16% of palmitic acid (16:0) and small amount of

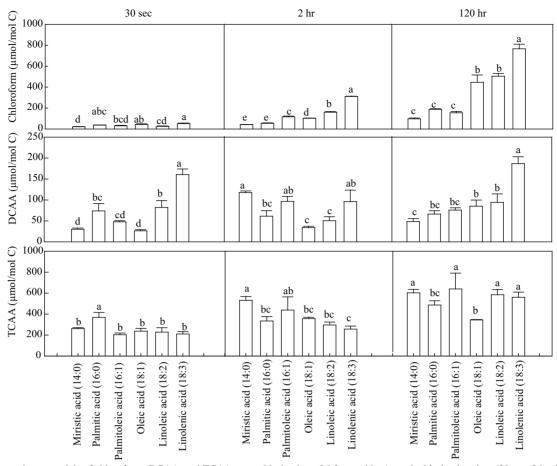


Fig. 1 Formation potentials of chloroform; DCAA, and TCAA upon chlorination of 6 fatty acids. At each chlorination time (30 sec, 2 hr, 120 hr), no significant difference (p > 0.05) was found among means with the same letter(s) on the top, according to one-way ANOVA. Error bars represent standard deviation.

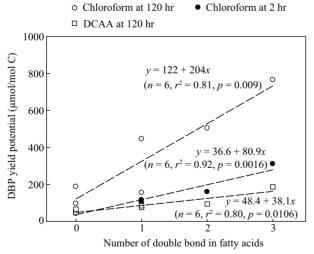


Fig. 2 Correlation between number of double bonds in fatty acids and generation potentials of chloroform at 120, 2 hr, and DCAA at 120 hr, respectively, upon chlorination of 6 fatty acids.

myristic acid (3%), oleic acid (2%), stearic acid (1%) and linolenic acid (1%) (Kates and Volcani, 1966). Therefore, the DBP (chloroform, DCAA and TCAA) yield upon chlorinating the algal lipid was calculated accordingly to the FAs distribution in algal lipid and DBPs formation potential of FAs observed in this study. In the calculation, extrapolations were performed for eicosapentaenoic and stearic acids based on the linear regressions in Fig. 2 to

obtain the corresponding DBP formation potential values, whereas those of palmitic, myristic, oleic and linolenic acids were employed directly.

The calculated DBP potential of diatom lipid was compared with the observed (Fig. 3), showing that the calculated levels were much lower, at most 30% of those observed. This suggested that aside from the fatty acids

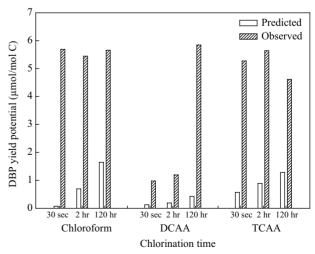


Fig. 3 Predicted and observed yield of DBPs upon chlorinating diatom lipid. Predicted yield was calculated base on the FA composition in the diatom lipid and the corresponding DBPs yield determined in the present study (Fig. 1). DBP yield of eicosapentaenoic acid (20:5) was extrapolated from regressions (Fig. 2).

present in the diatom lipophilic fraction that contribute at most 30% of the total DBP yield, other lipophilic substances such as lipoprotein might also be a potent DBP precursor. Another interpretation was that the FA composition of the diatom lipophilic fraction in this study was different from those obtained by Kates and Volkani (1966), because fatty acid composition was found to vary with algal growth stage and environmental conditions (Denicola, 1996; Hong et al., 2008a).

A comparison was also performed between the algal lipophilic fraction and the other algal fractions (algal cells, total, hydrophilic and hydrophobic proteins) in producing DBPs upon chlorination (Fig. 4). For chloroform, this showed that the algal lipophilic fraction and the hydrophilic protein served as "fast" and potent precursors, highly reactive right after chlorination (0.5 min) (algal lipophilic fraction: 1365 µmol/mol C; hydrophilic protein: 4466 µmol/mol C). The reactivity of the algal lipophilic fraction remained constant until the end of the chlorination at 2 hr (1358 µmol/mol C), while that of the hydrophilic protein increased more than two times in magnitude (9449 µmol/mol C). On the other hand, the formation of DCAA and TCAA from the algal cells was relatively constant throughout the chlorination, showing a similar pattern as that from the algal lipophilic fraction (Fig. 4).

Navicula cells are composed mainly of 23.4% protein, 10.3% of carbohydrates and 66.3% of lipid within the cell (Watson et al., 2005). Assuming the DBPs from chlorinating carbohydrates were negligible (Hong et al., 2008a), a calculation was conducted in order to compare calculated (predicted) DBP yields with those observed in the present study using data at 2 hr chlorination (Fig. 5). The predicted values for chloroform, DCAA and TCAA were less than 35% different from the corresponding observed values. This finding demonstrated that the algal community and the associated biochemical compositions in the source water served as an important bioindicator for a preliminary estimation of chloroform, DCAA and TCAA formation upon chlorination, particularly for raw water in which algal-derived organic matter is the dominant organic carbon such as in the case of Hong Kong's source water (Hong et al., 2008b). This finding should be helpful in designing organic carbon removal within water treatment plants. Yet, the other algal-derived organic materials, such as decomposed algal cells, should also be

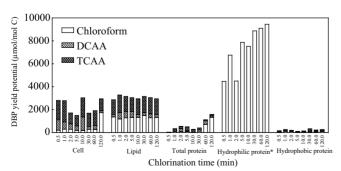


Fig. 4 DBPs formation potentials among algal-derived organic materials. * DCAA and TCAA were not detected in chlorinated hydrophilic proteins due to contamination of organic carbon (DCAA and TCAA precursors) during protein extraction.

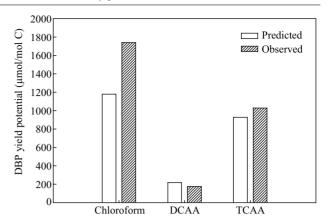


Fig. 5 Comparison of DBP yield potentials upon chlorination of algal organic materials at 2 hr chlorination, between theoretical calculated and laboratory experimental values.

taken into consideration when characterizing their role as DBPs precursors. Our future focus will be directed to this particular aspect.

3 Conclusions

Chloroform and DCAA production of the six fatty acids (FA) was found to depend on the degree of saturation of the FA. The high DBP yield of the diatom lipophilic fraction was contributed mostly by other lipophilic substance(s), while FAs contributed to less than 30% of the total DBP production. Comparison among different algalderived materials indicated that the lipophilic fraction was a more potent precursor of chloroform, DCAA and TCAA than proteins. There was no significant difference between the calculated and observed values of DBP production based on the three main biomolecules (lipid, protein, carbohydrates) in algal cells. This suggested that when algal-derived organic materials served as the main organic carbon in source water, the percentage of algal protein and lipid could be used for a preliminary estimation/calculation of chloroform, DCAA and TCAA formation upon chlorination.

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