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Formation of water disinfection byproduct 2,6-dichloro-1,4-benzoquinone from chlorination of green algae

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ABSTRACT

We report that green algae in lakes and rivers can serve as precursors of halobenzoquinone (HBQ) disinfection byproducts (DBPs) produced during chlorination. Chlorination of a common green alga, *Chlorella vulgaris*, produced 2,6-dichloro-1,4-benzoquinone (2,6-DCBQ), the most prevalent HBQ DBP in disinfected water. Under varying pH conditions (pH 6.0–9.0), 2,6-DCBQ formation ranged from 0.3 to 2.1 μg/mg C with maximum formation at pH 8.0. To evaluate the contribution of organic components of *C. vulgaris* to DCBQ formation, we separate the organics into two fractions, the protein-rich fraction of intracellular organic matter (IOM) and the polysaccharide-laden fraction of extracellular organic matter (EOM). Chlorination of IOM and EOM produced 1.4 μg/mg C and 0.7 μg/mg C of 2,6-DCBQ, respectively. The IOM generated a two-fold higher 2,6-DCBQ formation potential than the EOM fraction, suggesting that proteins are potent 2,6-DCBQ precursors. This was confirmed by the results of the chlorination of proteins extracted from *C. vulgaris*: the amount of 2,6-DCBQ produced is linearly correlated with the concentration of total algal protein (R^2 = 0.98). These results support that proteins are the primary precursors of 2,6-DCBQ in algae, and control of green algal bloom outbreaks in source waters is important for management of HBQ DBPs.

Introduction

Surface waters are an important source of drinking water globally. While algal blooms are a regular seasonal occurrence in most surface waters, the extent and duration of these blooms is increasing due to anthropogenic activities (Yang et al., 2008; Zhang et al., 2010a; Zhang et al., 2010b; Glibert, 2016). This poses a great challenge to the maintenance of drinking water quality, as algal blooms have been associated with clogged treatment plant pipes, odor and taste events, and toxicity events from toxin-producing blue-green algae (Knappe, 2004). Furthermore, increased algae and algal organic matter (AOM) in source waters has also been shown to affect the formation of drinking water disinfection by-products (DBPs) (Nguyen et al., 2005; Fang et al., 2010; Yang et al., 2011). AOM among source waters is derived from the metabolic activity of algae and is generally categorized as extracellular organic matter (EOM), products released by living

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cells, and intracellular organic matter (IOM), products of cell lysis (Li et al., 2012; Wert and Rosario-Ortiz, 2013).

The variation in biochemical composition between different species of algae and their fractions of AOM is well documented, with noted differences between numbers of unsaturated alkyl chains, carbohydrates, proteins, and other algal components (Brown et al., 1997; Pivokonsky et al., 2006). Thus, differences in DBP yield from algal precursors are often attributed to this biochemical variation and its resulting effects on DBP formation potential. For different phyla, *Nitzschia* sp. (diatom) showed higher chloroform yields (48 mg/mg C) but lower HAA yields (43 mg/mg C) than *Chlamydomonas* sp. (green algae) (chloroform: 34 mg/mg C; HAA: 62 mg/mg C) and *Oscillatoria* sp. (blue-green algae) (chloroform: 26 mg/mg C; HAA: 72 mg/mg C) (Hong et al., 2008). As for the same phylum, Huang et al. (2009) found that *Anabaena flos-aquae* were in the range of 2–11 μmol/mmol C for THM and 2–17 μmol/mmol C for HAA, while those of *Microcystis aeruginosa* were slightly higher.

Among the biochemical composition, proteins or amino acids generally represent the largest fraction of organic compositions for many algal species, particularly for cyanobacteria and green algae, with a protein fraction ranging between 70% and 36% on the basis of total dry weights (Kirpenko et al., 2016; Huang et al., 2016). Furthermore, the IOM fraction contained significantly greater portion of proteins than the EOM. The differences in the production of proteins also depend on the species, its growth phase, the age of the culture, and on the culture conditions (Pivokonsky et al., 2006; Henderson et al., 2008). As a result, the presence of algae can dramatically affect the characteristics of the amino acids in natural waters. Scully et al. (1988) examined the formation potential of THMs from chlorinated lake waters and found algal proteins contributed to roughly 10% of the measured THM formation potential. Another study from Selbes et al. (2015) examined DBP formation from chlorination of nine amino acids and found that aspartic acid and histidine produced high amounts of dihalo-HANs and dihalo-HAAs. But, most studies that examined DBP formation potential from proteins and free amino acids have focused on the formation of C-DBPs (*e.g.*, THMs and HAAs) and certain N-DBPs (HANs and halonitromethanes (HNMs)) (Chu et al., 2010; Ge et al., 2011; Le et al., 2016). The relative contribution of different algal biochemical components to the formation of halobenzoquinone (HBQ) DBPs, however, has not been elucidated.

HBQs are an emerging class of DBPs that have been predicted to be likely carcinogens based on quantitative-structure toxicity relationship (QSTR) analysis (Qin et al., 2010; Bull et al., 2011; Yang and Zhang, 2013). The predicted chronic lowest adverse effect levels (LOAELs) for HBQs were estimated to be four orders of magnitude less than those of the regulated THMs and HAAs. In addition, recent cytotoxicity and genotoxicity studies have shown that HBQs can cause DNA damage in T24 human bladder cancer cells and *Escherichia coli* (Du et al., 2013; Chen et al., 2015). Four HBQs were initially discovered as DBPs at levels ranging from 0.5 to 165 ng/L in drinking water (Zhao et al., 2012). These included 2,6-dichloro-1,4-benzoquinone (2,6-DCBQ), 2,3,6-trichloro-1,4-benzoquinone (TriCBQ), 2,6-dibromobenzoquinone (2,6-DBBQ), and 2,6-dichloro-3-methyl-1,4-benzoquinone (DCMBQ). Among these four, 2,6-DCBQ is the most frequently detected HBQ and is found at the highest concentrations in both drinking water and recreational waters (Diemert et al., 2013; Wang et al., 2013a) Although phenol has been reported as a precursor (Zhao et al., 2012), the formation of HBQs from algal precursors has not been examined.

The objective of the current study is to determine if algal precursors can produce HBQ DBPs during chlorination. *Chlorella vulgaris*, a commonly found green alga in surface water (Przytocka-Jusiak, 1984; Krienitz et al., 2015), was chosen as the target alga, and 2,6-DCBQ was selected as a typical HBQ due to its high occurrence frequency and abundance among HBQs (Zhao et al., 2010; Wang et al., 2014). Specifically, this study aims to (1) examine the formation of 2,6-DCBQ from *C. vulgaris* during chlorination; (2) compare 2,6-DCBQ yield from fractionated AOM (IOM and EOM fractions); and (3) determine the role of protein in 2,6-DCBQ formation.

1. Materials and methods

1.1. Materials

C. vulgaris (FACHB-6) was obtained from the Freshwater Algae Culture Collection of the Institute of Hydrobiology, Chinese Academy of Sciences. 2,6-DCBQ with a purity greater than 98% was obtained from TCI (Tokyo Chemical Industry Co., Ltd.). Acetonitrile and formic acid (FA) were purchased from CNW Technologies (Shanghai ANPEL Scientific Instrument Co., Ltd.; 98.0%). Surrogate algal biomolecules were obtained as follows: bovine serum albumin (BSA) from AOBOX Biotechnology Co., Ltd. (Beijing), fish oil (commercial Alaska fish oil) from Nu-Health Products Co. (Walnut, CA), and starch from TianJin Kemiou Chemical Reagent Co., Ltd. (Tianin City, China). Stock solutions of chlorine were prepared by diluting a commercial solution of sodium hypochlorite (NaClO, 9% active chlorine). All other chemicals were reagent grade or higher and used without further purification. All solutions for this study were prepared with Milli-Q water (Mill-Q SP VOC, Millipore Co., Bedford, MA).

1.2. Algae cultivation

C. vulgaris was maintained in OECD media (pH 8.0) according to the Organization for Economic Co-operation and Development (OECD) guideline (OECD, 2006). The OECD media contained the following ingredients: $FeCl_3{\cdot}6H_2O$ (80 μg/L), $CaCl_2{\cdot}2H_2O$ (15 mg/L), $MgSO_4{\cdot}7H_2O$ (15 mg/L), $MgCl_2{\cdot}6H_2O$ (12 mg/L), $Na_2EDTA{\cdot}2H_2O$ (0.1 mg/L), $ZnCl_2$ (3 μg/L), $CuCl_2{\cdot}2H_2O$ (0.01 μg/L), $MnCl_2{\cdot}4H_2O$ (0.415 mg/L), $CoCl_2{\cdot}6H_2O$ (1.5 μg/L), $Na_2MoO_4{\cdot}2H_2O$ (7 μg/L), H_3BO_3 (0.185 mg/L), NH_4Cl (15 mg/L), KH_2PO_4 (1.6 mg/L). All cultures were maintained in a SPX-250B-Z incubator (Shanghai Boxun Industry & Commerce Co., Ltd., China) under a 12 hr light/12 hr dark regime at 25 ± 0.5°C, with illumination provided by a 2500 lx fluorescent lamp. Algae samples for fractionation and for chlorination tests were collected during exponential growth phase after four days of cultivation.

1.3. Extraction of EOM and IOM

After collection, cultivated algae were centrifuged at 10,000 r/min for 10 min. The supernatant was then collected and filtered

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