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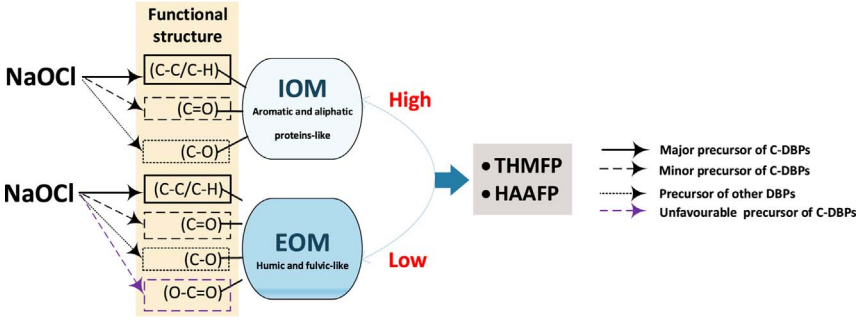
Chemical structures of extra- and intra-cellular algogenic organic matters as precursors to the formation of carbonaceous disinfection byproducts

Lap-Cuong Hua[a], Jr-Lin Lin[b], Pei-Chung Chen[a], Chihpin Huang[a,*]

[a] Institute of Environmental Engineering, National Chiao Tung University, Hsinchu, Taiwan, ROC
[b] Department of Environmental Engineering, Chung Yuan Christian University, Taoyuan, Taiwan, ROC

GRAPHICAL ABSTRACT



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ABSTRACT

Algal eutrophication in reservoirs is frequently accompanied by a remarkable increase in the concentration of algogenic organic matter (AOM). Because AOM is well-known as an important precursor to disinfection byproducts (DBPs) in drinking water, algal eutrophication poses severe concerns for public health. This study aimed to characterize the chemical properties of AOM from two major origins, i.e. extra- (EOM) and intracellular organic matter (IOM), of a commonly found green alga *Chlorella* sp. as precursors to trihalomethanes (THMs) and haloacetic acids (HAAs). Particularly, the corresponding relation between the chemical functional structure of EOM and IOM and their THM and HAA formation potential (THMFP and HAAFP) was comprehensively investigated. Results show that IOM chiefly comprised of aromatic and other aliphatic protein-like materials (high organic nitrogen content) those have high activity for chlorine substitution (high UV_{253}/UV_{203} value) due to the high hydroxyl and amide contents in its chemical structures. EOM alternatively exhibited the characteristics of humic- and fulvic-like materials accompanying with high DOC/DON ratio and aromaticity. However, the chemical structure of EOM had a low tendency for chlorine substitution (low UV_{253}/UV_{203} value); it was also accompanied by a considerable content of carboxylic moisture, which likely resulted in an unfavorable substitution reaction of EOM with chlorine. As a result, the carbonaceous DBP formation potential (C-DBPFP) showed that IOM yielded higher levels of both THMFP and HAAFP than that yielded by EOM.

1. Introduction

Eutrophication of algae in reservoirs frequently impedes the operational performance of water treatment plants (WTPs) because of the exponential rise in cell population and the concentration of algogenic organic matter (AOM) in the water column [1]. When AOM enters

* Corresponding author.
E-mail address: cphuang@mail.nctu.edu.tw (C. Huang).

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the water system, this autochthonous organic source contributes a substantial amount of organics to the natural organic matter (NOM) pool [2]. The AOM released by various algae species can contribute as much as 50% of total dissolved organic carbon (DOC) in surface water during low rainfall seasons [3]. It correspondingly causes drastic changes in the amount of organic matter in treated water [4]. Critically, AOM has been well-proven as an important organic precursor to the formation of organic halogenated DBPs, such as trihalomethanes (THMs) and haloacetic acids (HAAs) [5–7], which are widely regulated due to their carcinogenicity. In the chlorination of natural water, THMs and HAAs are two prevalent organic halogenated DBPs derived from AOM and account for more than 50% by weight of total organic halides [8–10]. Besides, chlorination of AOM derived from green algae (*Chlamydomonas* sp.) or cyanobacteria (*Microcystis* sp.) could result in the formation of other DBPs, such as haloacetaldehydes, haloacetonitriles, or chloropropanone [11,12]. Because they are carcinogenic for humans, halogenated DBPs in finished drinking water have posed severe concerns for public health [13].

In natural water, AOM plays as an important autochthonous organic input from algae; its characteristics are manifest differentiated from terrestrial NOM [5,14]. AOM comprises low aromaticity with high organic nitrogen content and is mainly hydrophilic [5,7,9,15]. Because of its nature, AOM is more difficult to be destabilized by traditional coagulation methods compared to NOM [1,16,17]. AOM thus has potential to enter post-chlorination acting as the precursors for the formation of DBPs, which then intensifies the formation potential of halogenated DBPs.

The origin of AOM can be classified into extracellularly soluble organic matter arising from the metabolism or excretion of algal cells (i.e. extra-cellular organic matter – EOM) and intra-cellular organic matter (IOM), resulting from cell autolysis. These origins contribute to different compositions of AOM, correspondently determining the category and level of DBP formation in the chlorinated water [7,9,10]. In general, IOM has been reported as a higher-yielding precursor to THMs and HAAs than that of EOM. This tendency is mostly true for various algal species, such as *Scenedesmus* sp., *Microcystis aeruginosa*, or *Cylotella*. [7,9,18,19]. Graham [18] showed that the chlorination test for the diatom *Asterionella formosa* and cyanobacteria *Anabaena flos-aquae* could produce three and fivefold higher THM concentrations compared to those produced by their corresponding EOM. Plumer and Edzwald [19] reported that the organic matter derived from diatom *Cyclotella* cells (or IOM) significantly contributed as much as 73% of total chloroform and 71–75% of total HAAs.

This study aimed to characterize the chemical properties of two AOM origins, EOM and IOM, derived from *Chlorella* sp. as precursors to THMs and HAAs. *Chlorella* sp. was selected as testing algal species in this study due to that it has been found as a dominant species in several shallow eutrophic lakes in the offshore of Taiwan. This phenomenon has caused massive problems toward the local WTPs, especially for DBP control. Other occasional eutrophication of *Chlorella* sp. was also reported in some shallow eutrophic lakes in China, India, and Turkey [20–23]. Particularly, *Chlorella* sp. was found to be the dominantly blooming species (accounting for 96.7%) and caused drastic fish kill in Kokulimidu Lake (Indian) [23]. It was also found as dominant species in shallow Cernek Lake (Turkey) [22] or Baiyangdian and Nansi Lake (China) [20,21]. Compared to other commonly blooming algae, i.e. *Microcystic* sp., however, there has very limited information regarding the role of *Chlorella* sp. derived organic matter on the formation of DBPs, especially HAAs (as seen in Table S1). Because AOM characteristics vary with algal species, this is crucial to give more insight into the characterization of AOM from *Chlorella* sp. as precursors to DBPs.

For an effective control of DBP formation in the treatment of drinking water treatment, a number of researchers have tried to characterize the chemical nature of AOM and its corresponding DBP formation potential (DBPFP) by using different qualitative methods, such as UV–visible absorbance, fluorescence excitation-emission matrix (EEM), and solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy [5,7,9,14,24]. Specific ultraviolet absorbance at 254 nm (SUVA value) is commonly used as a surrogate of aromaticity for either AOM or NOM and their DBPFP, because of its simplicity and well correlation to DBPFP [5,25,26]. The other useful UV indicator for predicting DBPFP is the UV_{253}/UV_{203} ratio [27,28]. Kroshin [27] proposed that this value can represent the reactivity of carbon sites (or activated carbon site) in aromatic moisture and is a good surrogate for THMFP prediction. Fluorescent spectroscopy (EEM) is also a sensitive approach for organic matter characterization and DBP precursor prediction [29]. By making a correlation between regional fluorescent intensities with DBPs, Yang [30] determined that the cumulative EEM spectra of aromatic protein-like and humic-like substances could be used to predict the yields of dichloroacetic acid (DCAA), chloroform, dichloroacetonitrile (DCAN), and total organic halide (TOX). Compared to UV–vis and EEM, solid state NMR spectroscopy (^{13}C NMR) is a more advanced method for quantitative characterization of the detailed chemical structures of AOM. Although the characterization of AOM has been rapidly investigated, there is still much to be learned about its chemical structures with the formation of DBPs.

In this study, the chemical functional structure of EOM and IOM derived from *Chlorella* sp. was studied by X-ray photoelectron spectroscopy (XPS) accompanying with NMR spectroscopy and other basic analysis. To the best of our knowledge, it is the first time that an investigation of the chemical functional groups of IOM and EOM by XPS has been carried out. Notably, the XPS results were used to correlate with THMFP and HAAFP, thus providing a deeper insight into the physicochemical characteristics of EOM and IOM, and leading to a better understanding of the nature of AOM origins and their relationship to C-DBPs.

2. Material and methods

2.1. Algal cultivation and AOM isolation

An axenic culture of the green algae *Chlorella* sp. was conducted in a 5 L photobioreactor containing an artificial medium (modified Chlorella medium) (pH 7.4) with the following constituents (per litter) 1.011 g KNO_3, 0.621 g NaH_2PO_4, 0.089 g Na_2HPO_4, 0.298 g $MgCl_2{\cdot}6H_2O$, 0.0113 g $CaCl_2$, 0.0186 g EDTA, 0.0139 g $FeSO_4{\cdot}7H_2O$, 6.2 mg H_2BO_3, 16.9 mg $MnSO_4{\cdot}H_2O$, 28.7 mg $ZnSO_4{\cdot}7H_2O$, 0.25 mg $CuSO_4{\cdot}5H_2O$, and 1.29 mg $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ The algal strain was isolated from Sheng-Li reservoirs on Matsu Island, Taiwan. The culture medium was sterilized by autoclaving at 121 °C for 30 min prior to use. Algal cultivation was carried out at room temperature (25 ± 1 °C) with intermittent illumination (16:8 light-dark cycle) by TL 5 lamps (35 μmol photons/m^2-s). Illumination was measured with a LI-250A Light Meter with a LI-190R quantum sensor (LI-COR, Inc., Lincoln, Nebraska, USA). Aeration at a rate of 500 mL/min was applied for rapid algal growth. Chlorophyll *a* content and optical density (OD) at 680 nm were monitored to determine the growth phase.

For AOM isolation, late exponential phase of algal growth (young and healthy cells) was selected to eliminate the contamination of EOM samples from the released IOM of senescent cells during the stationary or declined phases. Algal suspension was centrifuged for 15 min, at 2700 G, at 4 °C (by Boeco Centrifuges U-320R, Germany) to separate algal cells from the medium. The supernatant was filtered through a 0.45 μm mixed cellular membrane filter (Advantec, Japan). The resulting filtrate was denoted as EOM. The remained algal pellets were washed twice with deionized (DI) water and consecutively lyophilized by a free-drier (Pin Chen Co., Taiwan) at −50 °C and $2{\cdot}10^{-4}$ tor for 6 h. These lyophilized algal cells were further physically ground for 2 min with a mortar and pestle. After that, the ground cells were resuspended in DI and mixed by a vortex mixer for 30 s to extract the IOM solution from the ruptured cells. This suspension was then centrifuged for 15 min, at 2700 G, and at 4 °C, and the supernatant was filtrated