EMERGENCE AND FATE OF IODINATED ORGANIC COMPOUNDS AND
DISINFECTION BY-PRODUCTS DURING BIOLOGICAL
TREATMENT OF OIL AND GAS
PRODUCED WATER

by
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A thesis submitted to the Faculty and the Board of Trustees of the Colorado School of Mines in partial fulfillment of the requirements for the degree of Master of Science (Civil and Environmental Engineering).

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ABSTRACT

Oil and gas (O&G) development in arid regions throughout the United States has increased water demands for development of industrial, agricultural, and residential sectors. Treating and recycling wastewater generated during O&G production for potential surface discharge and beneficial reuse can help alleviate water demands in several water-intensive sectors. Several treatment technologies to improve water quality for potential surface discharge, live-stock watering, dust suppression, and on-site water reuse can be implemented. Biological active filters (BAFs) are one treatment technology that can be effectively used as pre-treatment of O&G wastewater to remove organic matter and reduce fouling in downstream membrane treatment processes for desalination. Because O&G wastewater is halogen rich, formation and toxicity of treatment byproducts with iodide and bromide constituents is of concern when planning potential treatment management strategies for complex waste streams like O&G produced water.

In this study, we investigated the occurrence of iodinated organic compounds (IOCs) in BAFs treating O&G produced water. The occurrence of three IOCs was monitored by quantifying chloroiodomethane, diiodomethane, and triiodomethane in nine BAF treatment systems operated with different granular activated carbon media and nutrient type before and after treatment. Chloroiodomethane, diiodomethane, and triiodomethane were measured by headspace solid-phase microextraction gas chromatography mass spectrometry at concentrations up to 16.6 µg/L, 442 µg/L, and 4,316 µg/L, respectively. Triiodomethane, an iodinated disinfection byproduct (I-DBP), was the IOC that was predominantly measured in treated produced water with more than 90% contribution to the total sum of three quantified IOCs in 21 samples analyzed (n=21). A moderately strong correlation (r=0.59) was established between iodide concentration and the total concentration of the three quantified IOCs (n=26). This relationship indicates the likelihood that the inorganic iodide introduced to the system in PW is converted to IOCs (organic iodine) during treatment. Additionally, organisms belonging to the iodide oxidizing bacterium (IOB) genus were also found at relatively high abundance (51.5%) in water treated through biological active filters but not produced water (0.2%). The occurrence of IOB, IOCs, and I-DBPs during biological treatment of O&G produced water has not been previously reported and can be indicative of an underestimated formation pathway of I-DBPs in complex waste streams.

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O&G	Oil and gas
BAFs	Biological active filters
I-DBPs	Iodinated disinfection byproducts
IOB	Iodide oxidizing bacteria
NSF	National Science Foundation
SRN	Sustainable Research Network
WE^{2}ST	Water-Energy Education, Science and Technology
AQWATEC	Advanced Water Technology Center
FB	Flowback
PW	Produced water
DOC	Dissolved organic carbon
PAH	polycyclic aromatic hydrocarbons
DJ	Denver Julesburg
PVC	Polyvinyl chloride
KMnO$_4$	Potassium permanganate
GAC	Granular activated carbon
EC	Electrical conductivity
HPLC	High performance liquid chromatography
SPME	Solid-phase microextraction
MSD	Mass spectrometer detector
PTV	Programmed temperature vaporizing
SRM	Selected reaction monitoring
SIM	Selected ion monitoring
HS	Headspace
GC	Gas chromatography
MeOH	Methanol
DNA	Deoxyribonucleic acid
PDMS/DVB	Polydimethylsiloxane/Divinylbenzene
PTFE	Polytetrafluoroethylene
IC	Ion chromatography
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
MDL	Method detection limit
MQL	Method quantification limit
DIN	Deutsches Institut für Normung (German institute for standardization)
TN	Total nitrogen
n	Number of samples in a set
H$_2$O	Water
CH$_2$ClI	Chloroiodomethane
CH$_2$I$_2$	Diiodomethane
CHI$_3$	Triiodomethane
CHClI$_2$	Chlorodiiodomethane
CHBrI$_2$	Bromodiiodomethane

ACKNOWLEDGMENTS

CHAPTER 1

INTRODUCTION

1.1 Oil and gas (O&G) wastewater production

Developments of new oil and gas (O&G) extraction technologies such as hydraulic fracturing (fracking) in conjunction with increased energy demands have enhanced production of O&G. Fracking as a well completion/stimulation technique is a water-intensive process and requires several million liters of water to fracture a single well [1-3]. Over the lifetime of a well, 10-70% of the water pumped into the well to fracture the rock formation returns to the surface as flowback (FB) and produced water (PW) [4]. FB and PW contain a variety of drilling fluid chemicals (e.g., proppants, friction reducers, surfactants, biocides, etc.), dissolved organic matter, metals, naturally occurring radioactive material, volatile and aromatic hydrocarbons, dissolved gases, and high concentrations of salt that can exceed that of seawater [3, 4]. Organic contaminants such as polycyclic aromatic hydrocarbons (PAHs), n-alkanes, polyethylene glycols, and polypropylene glycols can be present at concentrations ranging from several μg/L to mg/L [5, 6]. Thus, there is a growing concern of introducing persistent, mobile, and toxic chemicals derived from O&G activities into natural environments (e.g., groundwater, streams, wetlands) during reuse.

With global energy demands expected to grow 28% until 2040 [7], fracking provides the technology and economic means to exploit unconventional O&G supplies to meet this demand. Although many environmentally friendly alternatives to hydraulic fracturing are being developed (e.g., liquefied petroleum gas, Dry Frac, etc.), economic and infrastructure feasibility will continue to require fracking as the main well stimulation method [3] increasing demand for water resources and producing waste streams in the years to come.

1.2 O&G wastewater management

Currently, O&G wastewater management strategies are mostly inclined towards injection into Class II disposal wells due to economic reasons. Yet, increasing seismic activity near injection well sites has increased the necessity to reduce this management practice and shift towards potential treatment and reuse [8, 9]. Reuse of PW requires thorough economic and water

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quality evaluations because various anthropogenic and geogenic organic compounds in O&G wastewater can be persistent and toxic (e.g., polycyclic aromatic hydrocarbons, barium, benzene, etc.), with the potential of causing long- and short-term impacts on human health and the environment. Water reuse for irrigation, streamflow augmentation, well drilling, and dust suppression can be achieved only following partial or full treatment to remove contaminants of emerging concern [8, 10]. Yet, treatment for reuse can be challenging because of the variable chemistry of the wastewater generated from different shale plays and throughout the lifetime of a well that might require special pre-treatment.

For membrane treatment processes, pre-treatment options to reduce organic matter are often necessary to reduce membrane fouling potential. Biological treatment of O&G PW has shown substantial removal of organic matter, increasing O&G wastewater quality and preparing it for downstream membrane treatment and desalination before potential reuse [11-15]. Studies by Freedman et al. [11] and Riley et al. [12, 16] have demonstrated 75-90% removal of dissolved organic carbon (DOC) from PW and fracking FB using biological active filtration (BAF). In these studies, biofilm acclimation and high performance of BAF with O&G wastewater of different chemistries showed the robustness and flexibility of this technology for O&G water reclamation.

Degradation of organic matter can be achieved through complete mineralization of organic compounds or through various transformation pathways, which can result in byproducts [17]. Many of these byproducts (e.g., napthalene to *cis*-napthalene dihydrodiol [18]) can be more toxic than the parent compounds, raising concerns with respect to the innocuousness and sustainability of biologically treated waste streams. Thus, more attention to the non-biodegradable organic fraction as well as byproducts formed during treatment is required to understand the environmental implications when reusing and releasing FB and PW derived waste streams into the environment.

1.3   Occurrence of halides in O&G wastewater

Iodide is an element in the halogen group, and it is found primarily in marine environments as well as in O&G PW. It is an essential nutrient for synthesizing thyroid hormones in humans [19, 20], enzymatic activities in microorganisms [21-25], and an important component in x-ray imaging medical procedures (e.g., iodinated x-ray contrast media) [26].

Enzymatic and iodide methylation [27] biotransformation of halogenated metabolites has been observed in surface and marine environments. These biotransformation processes occur through metabolic activities of bacteria, fungi, marine algae, and other terrestrial organisms [24, 28]. Volatile organic iodine species such as diiodomethane and chloroiodomethane have been shown to be of biogenic origin, formed by biological reactions of iodide oxidizing bacteria (IOB) and other microorganisms [27-30].

Iodide can be found in multiple oxidation states and its speciation, bioavailability, and mobility are dictated by biological interactions and physical and chemical environmental conditions. Iodide is bioavailable in the form of I^- [21] and was only measured as I^- in PW used in this study. Yet, the concentration of total iodide species in the environment is expected to be mostly iodate (IO_3^-), iodide (I^-), and organic iodine [31]. Marine environments account for a substantial portion (70%) of the global iodide present in natural environments and is associated with microbiological activities with high volatile organic iodine release into the atmosphere [28, 31]. Iodine in seawater can be found at up to 45 to 60 μg/L levels, whereas in fresh water it is less than 10 μg/L [31]. Similarly, bromide is present in natural streams in the form of Br^- (-1 oxidation state) and is abundant in marine water at levels around 65 mg/L Br^- and in surface streams at concentrations ranging from 5 to 150 μg/L [32, 33].

The upstream O&G sector has experienced an increase in wastewater generation containing high concentrations of halides. Bromide and iodide present O&G wastewater is likely of geogenic origin from contact with ancient marine shale formations. In the Denver-Julesburg (DJ) basin and the Barnett shale, iodide have been measured at concentrations up to 40 and 53 mg/L I^-, respectively, while bromide has been measured at concentrations ranging from 125 to 172 mg/L Br^- [6, 11, 34].

Halide concentrations in PW have been measured at elevated concentrations [34] and can interact with and transform organic matter. Thus, more information about precursors of iodinated organic compounds (IOC) and iodinated disinfection byproducts (I-DBPs) in wastewater with high organic matter and halide concentrations is needed to determine potential mitigation strategies and ensure safe water reuse applications.

IOCs are organic compounds that contain one or more carbon–iodine bonds. The majority of IOCs feature iodide connected to one carbon center. Iodoform, also referred to as triiodomethane (CHI_3), and diiodomethane (CH_2I_2) used to be industrially relevant IOCs due to

their antiseptic properties. Diiodomethane in its liquid form is also a valuable industrial chemical because of its high density, which is used to determine the density of solids and minerals [35], and it is also used as an optical contact liquid for surface hydrophobicity characterization [36, 37]. Choloroidomethane (CH_2ICl) and diiodomethane are also know to form biotically by iodide oxidizing bacterium [27] and other marine microorganisms. Triiodomethane, chlorodiiodomethane, and bromodiiodomethane are also I-DBPs that forms by haloform reactions, where organic matter is halogenated through nucleophilic substitutions and eliminations. Emerging research has shown the presence of halogenated organic compounds in O&G wastewater, including 20 IOCs [38] and the formation of IOC such as I-DBPs during disinfection (chlorination and chloramination) of water impacted by iodide-rich O&G wastewater [34, 39]. This has raised concerns regarding the fate of O&G wastewater because I-DBPs are more cytotoxic and genotoxic [34, 38-42] than their brominated and chlorinated analogs, with detrimental environmental impacts and adverse health effects at sub µg/L levels [34, 38, 40-44].

1.4   Public relevance and broader impacts

In the DJ basin in northeastern Colorado most water used for fracking operations comes from surface water and ground water sources [45]. This region also experiences high demands from industrial, residential, and agricultural sectors. Reuse is a compelling alternative to decrease water stress in many fracking intense regions like the DJ basin. In order to consider beneficial water reuse, factors such as reuse applications and regulatory frameworks must be considered. Water discharge for surface water stream augmentation is a concern when managing highly impaired streams such as O&G wastewater. Discharge permits issued by the Water Quality Control Division can cover a variety of effluent limitation guidelines for treatment facilities discharging O&G produced water [46]. Yet, there are still uncertainties in regards to unknown contaminants in wastewater.

Recently, preliminary effluent limits set forth by the Colorado's Department of Public Health and Environment for release of partially treated O&G produced water into surface streams in the Greater Wattenberg Area do not address limits on organic matter constituents such as DOC, hydrocarbons, surfactants, biocides, scale inhibitors, dissolved methane, and other classes of organic contaminants [46]. Such regulatory limits are not in place due to the lack of

information regarding organic constituents in O&G wastewater. Currently, few studies characterizing organic contaminants in O&G wastewater can be found in literature [5, 6, 47-51]. Yet, studies analyzing the fate of discharged treated O&G wastewater into surface streams suggest that this can be problematic if this water is used in downstream drinking water treatment facilities due to disinfection of water containing significant halide concentrations [39].

1.5 Research objective

In this study, the emergence and fate of three iodinated organic compounds (IOC)—chloroiodomethane, diiodomethane, and triiodomethane—were investigated during biological treatment of O&G PW using headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME-GC-MS). Qualitative and quantitative analytical approaches were employed to establish relationships between the occurrence of IOCs in BAF effluent and different operating conditions (e.g., media type, nutrient addition). Furthermore, the samples analyzed during stage III were re-examined 137 days after collection to understand the fate of IOCs after prolonged storage. The presence of IOB in the aqueous phase as suggested by other studies was also evaluated. This study was focused on understanding potential formation pathways of halogenated DBPs that can support the development of mitigation strategies.

CHAPTER 2

MATERIALS AND METHODS

2.1 Biological active filter (BAF) system

Water samples for this study were obtained from a bench-scale BAF system consisting of nine biological active filter columns. In brief, columns were constructed with clear polyvinyl chloride (PVC) pipe (5 cm inner diameter, 147 cm length, 76 cm media depth) and were connected to individual 10 L storage tanks using clear polyethylene tubing. Columns were connected to a peristaltic pump and operated in up-flow, batch configuration as shown in Figure 2.1. Three sets of three columns were filled with three distinct granular activated carbon (GAC) media as specified in Table 2.1 (pg. 9). Details regarding column optimization and specifications can be found in Riley et al. [12].

Figure 2.1. Schematic of a set of three BAF columns operated independently. Arrows indicate flow direction. Sampling location was at the top of the column before returning to the storage tank.

2.2 BAF system operation

The BAF system was operated continuously over a 13-week period with constant replenishment of produced water at 72-hr intervals to assess the occurrence of IOC during long-term operations.

2.2.1 Experiment set up

Raw PW (referred to as BAF influent or influent in this thesis) obtained from the DJ Basin (Colorado, United States) was oxidized with 10 mg/L KMnO$_4$ at least 24 hours prior to each experiment to allow for oxidized solids to settle. Each column was continuously aerated (10 mL/min) at the inlet of the columns and operated in batch mode at 2.44 m/h (83 mL/min) hydraulic loading rate with approximately 10 L of raw O&G PW. Columns were operated in parallel over a 13-week period with a new batch of produced water treated at 72-hr intervals. After 72 hrs of treatment, effluent was collected immediately after passing through the filter, columns were backwashed with chlorinated tap water (50% bed expansion), and each column was replenished with fresh PW.

2.2.2 Sample collection

One BAF influent sample was collected before treatment while BAF effluent samples were collected after 72 hrs of treatment at three treatment stages: at week 4 (stage I), week 8 (stage II), and week 13 (stage III) after starting the system (i.e., week 1 of each stage). Duplicate samples were collected without headspace in 40 -mL amber vials and stored at 4 °C until analysis (less than one week). Dissolved organic carbon (DOC), pH, electrical conductivity (EC), IOCs concentrations, and iodide concentrations were measured at every sampling stage whereas major anion and cation constituents were only measured during stage I. Additionally, samples were stored with approximately 5 mL of headspace, with no preservatives or adjustments, and were analyzed after 137 days of storage to re-evaluate the fate of IOCs.

2.3 General chemistry

A comprehensive analysis of PW chemistry was conducted throughout the study to identify the potential impacts of water chemistry on target compounds.

2.3.1   Produced water quality analysis

DOC was analyzed using a Shimadzu TOC-L analyzer (Columbia, MD). Samples were filtered using 0.45 µm polypropylene filters and acidified (pH≤2) with hydrochloric acid. Samples with DOC exceeding 100 mg/L DOC were diluted (1:10) with ultrapure water. Samples were analyzed for iodide concentration, EC (Cole Parmer, Vernon Hills, IL), and pH (VWR, Radnor, PA) using calibrated handheld probes at room temperature (20 °C). For iodide concentrations, samples were diluted 1:20 with ultrapure water. Samples were analyzed using an iodide double-junction ion-selective electrode (Cole Parmer) and concentrations calculated using a five-point calibration (0.1, 1, 5, 10, 50 mg/L I^-) made by dilution of a 1000 mg/L I^- stock solution (Cole Parmer). Analysis of major cations and anions was conducted using an ICP-AES (5300DV, Perkin Elmer, Waltham, MA) and Dionex ICS-900 (Thermo Scientific, Waltham, MA), respectively. Samples were diluted 1:50 (Cl^- < 300 mg/L) and 1:60 (Cl^- < 100 mg/L) for ICP-AES and IC, respectively, to prevent chloride instrument saturation.

2.3.2   Nutrient addition and analysis

During each experiment, nutrients were dosed daily at a concentration of 21 mg/L nutrient solution 1 or nutrient solution 2 for the first 4 weeks, then 7 mg/L nutrient solution 1 or nutrient solution 2 in the last 5 weeks. Each nutrient type contained different macro- and micro-nutrient constituents (e.g., carbon, nitrogen, and phosphorous). Media and nutrient specifications for the nine individual BAF columns investigated are summarized in Table 2.1. In brief, each set of three columns was operated with the same GAC. One of the three columns was spiked with nutrient solution 1, one with nutrient solution 2, and one was kept as control without the addition of nutrients. C:P:N ratios of the nutrient solutions were calculated based on measured DOC, total nitrogen (TN), and total phosphorous (TP). For each nutrient, at least 90% of the total phosphorous (0.3 and 0.12 mg/L TP in 21 mg/L nutrient solution 1 and 2, respectively) was in the form of phosphate whereas the nitrogenous species were mostly ammonia and organic nitrogen (73% and 100% of TKN for nutrient solution 1 and nutrient solution 2, respectively). A C:N:P ratio for produced water was also calculated to be >500:63:1. This conservative ratio was derived assuming that the phosphorous concentration was at the method detection limits (MDL) (0.01068 mg/L P in ICP-AES) and adjusted for the dilution factor. Additionally, the nitrogen species were 75% ammonia (NH_3-N), 17.1% nitrite (NO_2^--N), and 7.9% organic nitrogen and

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nitrate (NO_3^--N) (of this 7.9% at least 3.2% is organic nitrogen as determined by a mass balance).

Table 2.1. Media and nutrient specifications for the BAF columns investigated in this study. All GAC medias were steam activated with different surface area and pore size distribution.

Name	Media type	Media Properties	Nutrient type	Nutrient properties
BAF 1	Norit® 816 (spent)[#]	Biofilm present	Nutrient 1	C:N:P = 4:7.5:1
BAF 2	Norit® 816 (spent)	Biofilm present	Nutrient 2	C:N:P = 13.4:10:1
BAF 3	Norit® 816 (spent)	Biofilm present	Control	
BAF 4	Norit® GAC 400*	Bituminous coal unwashed 12×40	Nutrient 1	C:N:P = 4:7.5:1
BAF 5	Norit® GAC 400	Bituminous coal unwashed 12×40	Nutrient 2	C:N:P = 13.4:10:1
BAF 6	Norit® GAC 400	Bituminous coal unwashed 12×40	Control	
BAF 7	Darco® 12×40 GAC*	Lignite coal acid washed 12×40	Nutrient 1	C:N:P = 4:7.5:1
BAF 8	Darco® 12×40 GAC	Lignite coal acid washed 12×40	Nutrient 2	C:N:P = 13.4:10:1
BAF 9	Darco® 12×40 GAC	Lignite coal acid washed 12×40	Control	

Peter D. Binney Water Purification Facility, Aurora Water, Aurora, Colorado
*Cabot Corporation, Alpharetta, GA

2.4 Analysis of iodinated organic compounds

IOCs were measured after 72 hrs of treatment using a headspace extraction method developed for the three compounds analyzed in this study.

2.4.1 Chemical reagents

Reagents used for chemical preparations were HPLC grade and were obtained from Fisher Scientific (Hampton, NH). Analytical grade diiodomethane (99%), triiodomethane (99%), chloroiodomethane (97%), triiodomethane-d (99%), and diiodomethane-d_2 (99%) were obtained from Sigma-Aldrich (St. Louis, MO) and chloroiodomethane-d_2 (≥98%) was obtained from Cambridge Isotope Laboratories, Inc. (Tewskbury, MA).

2.4.2 Chemical reagent preparation

Standard stock solutions of diiodomethane, triiodomethane, and chloroiodomethane in pure and deuterated form were prepared separately. Standard stock solutions (1 µg/µL) were made by adding 0.01 g of each compound into a 10 mL glass flask and diluted with methanol. Stock solutions were pipetted into 2 mL amber glass vials with no headspace, screw caps sealed with parafilm and stored in dark conditions at 4 °C to avoid photo degradation and evaporation.

2.4.3 HS-SPME-GC-MS

IOCs were analyzed by headspace solid-phase microextraction gas chromatography mass spectrometry (HS-SPME-GC-MS) using a polydimethylsiloxane/divinylbenzene (PDMS/DVB) fiber (Supelco, Bellafonte, PA). Solid-phase microextraction methods involved evaluation and determination of optimal extraction parameters for IOCs and the type of water investigated (PW is rich in volatile and semi-volatile hydrocarbons). For the initial testing we analyzed the fiber material followed by extraction factors that would yield acceptable reproducibility, resolution and reasonable extraction times. As depicted in Appendix A, SPME fiber material (Figure A1 (pg. 38)), extraction temperature and time (Figure A2 (pg. 39) and Figure A3 (pg. 40)), and desorption temperature and time (Figure A4 (pg. 41)) were selected based on the evaluation of extraction efficiency and standard deviation of all analytes. SPME sample preparation consisted of 5 mL of sample pipetted into a 20 mL GC-SPME amber vial covered with a 1.3 mm thick PTFE/silicone septa and magnetic screw cap, followed by the addition of deuterated internal standard stock solution. Extraction and sample preparation were conducted using the parameters summarized in Table 2.2.

Table 2.2. SPME extraction parameters

Parameters	Description
Fiber material	65μm (PDMS/DVB)*
Agitator speed	Sequential 250 RPM
Sampling depth	40 mm
Extraction time	(1) 60 min
	(2) 30 min
Extraction temperature	45 °C
Desorption time	3 min
Pre- and post-desorption temp	250 °C
Pre- and post-desorption time	1 min (2 min total)
Sample volume	5 mL
Headspace volume	15 mL
Internal standard	(1) 2 μL of 1 μg/μL in MeOH (C = 400 μg/L)
	(2) 25 μL of 10 μg/μL in H_2O (C = 50 μg/L)

*Supelco/Sigma Aldrich, St. Louis MO

Analysis was performed with a Trace 1310 gas chromatograph (Thermo Scientific, Waltham, MA) equipped with a capillary column and a split/splitless baffle liner (2 mm x 2.75 x 120) (Thermo Scientific, Waltham, MA). GC method details are summarized in Table 2.3.

Table 2.3. Gas chromatograph method parameters

Parameter	Description
Carrier gas	Helium
Flow	1.7 mL/min
Inlet mode	1:4 Split
PTV temperature	230 °C
Capillary column	1,4-bis(dimethylsiloxy)phenylene dimethyl polysiloxane Elite 5MS* or Rxi-5 Sil MS†
Column dimensions	(A) 30 m × 0.32 mm × 0.25 μm film thickness
	(B) 30 m × 0.25 mm × 0.25 μm film thickness
Oven temperature program	
Initial temperature; Hold time	40 °C; 1 min
Initial temperature ramp: hold time	5 °C/min to 60 °C held 2 min
Final temperature ramp:	10 °C/min to 220 °C

* Perkin Elmer, Waltham, MA
† Restek Corporation, Bellafonte, PA

The instrument was equipped with a Thermo Scientific TriPlus™ RSH autosampler and a TSQ™ 8000 Evo Triple Quadrupole mass spectrometer detector (MSD). The MSD was operated in full scan (mass range 45–500 m/z) electron-ionization mode (70 eV ionization energy). MSD method details are summarized in Table 2.4.

Table 2.4. Mass spectrometer detection parameters

Parameters	Description
Ion source	Electron Ionization (EI)
Source temperature	220 °C
Electron Energy	70 eV
Transferline temperature	250 °C
Emission Energy	50 μA
Scan range	45-500 Da

2.4.4 Identification and quantitation of iodinated organic compounds (IOCs)

Full scan mode was employed with the purpose of tracking other potential IOCs and transformation products in the water at any given stage of sampling. Total ion chromatograms (Figure 2.2) and extracted ion chromatograms were analyzed using Chromeleon 7.2 software (Thermo Scientific). Compound identification was achieved using mass spectra and retention times of analytical standards as well as reference database spectra (NIST standard reference database, version 2.2). Quantification of chloroiodomethane, diiodomethane, and triiodomethane

was achieved based on isotope dilution method or internal standard method using selected target ions and appropriate isotope-labeled analogues (Table 2.5 (pg. 13)). At least five calibrations solutions were prepared in both ultrapure water and BAF matrix (calibration standards concentrations were 1, 5, 25, 50, 100, 500, 1000, and 2500 µg/L) to establish matrix correction factors and adjust calculated concentrations accordingly. Correction factor details can be found in Table B.1 in Appendix B (pg. 42). Other volatile organic compounds that exhibited prominent peaks in the total ion chromatograms were identified based on their mass spectra and semi-quantified using internal standard method with diiodomethane-d_2 as the internal standard. Semi-quantification was achieved as described elsewhere [5] assuming a response factor of 1 with the internal standard.

Figure 2.2. Total ion chromatogram of (A) produced water (BAF influent from stage II) and (B) BAF effluent (BAF 3 week 13). Sample was enriched by extraction from the headspace of a 20-mL vial with 1:4 sample to headspace ratio.

2.4.5 Method detection and quantitation limits

Method quantification limits (MQL) and MDL for target analytes were calculated with risk evaluation according to DIN test 32645 [52]. Alpha (α) and a beta (β) values were estimated based on the smallest amount of sample with a 5% error and a type II error of the 50% probability that the instrument signal of the target compound is measured as a blank signal. Concentration of analytes detected above MDL but below MQL were estimated based on low range calibration curves. Although the analytical method can be further improved to achieve MDLs and MQLs in the ng/L range by using selected reaction monitoring (SRM) or selected ion monitoring mode (SIM), analysis in full scan mode was preferred to be able to also screen for other halogenated transformation products that were potentially formed during treatment. This expanded the scope of this study to both quantitative and qualitative identification of potentially hazardous compounds in PW after biological treatment. It should be noted that due to difficulties with the extraction of triiodomethane-d in BAF influent samples (raw PW), it was refrained from using triiodomethane-d as an internal standard for quantification. Triiodomethane was quantified using diiodomethane-d$_2$ as the reference internal standard in all samples and correction factors applied accordingly for concentration corrections. Analyte specific details including retention time, selected target ions, and detection and quantification limits are summarized in Table 2.5.

Table 2.5. Analytical parameters of three iodinated organic compounds. Note that retention times of each analyte are based on analysis on column A in Table 2.3.

Compound	Labeled Analogue	Primary m_1/z	Secondary m_1/z	Formula	MW [g/mol]	RT [min]	MDL [μg/L]	MQL [μg/L]
Chloroiodomethane	Chloroiodomethane-d$_2$	141 / 143	178 / 180	ClCH$_2$I	176.4	6.39 / 6.36	6.0	14.0
Diiodomethane	Diiodomethane-d$_2$	268 / 270	141 / 143	CH$_2$I$_2$	267.8	9.69 / 9.64	4.4	10.5
Triiodomethane	Triiodomethane-d	268 / 269	394 / 395	CHI$_3$	393.7	16.62/16.58	19.2	53.2

Abbreviations: MW- molecular weight. R$_t$ – retention time. m/z – quantifier ions. MDL- method detection limit. MQL- method quantification limit

Additionally, preliminary details of two additional compounds detected in BAF effluent after 137 days of storage and identified (Table C.1 (pg. 45)) as IOCs by reference mass spectral libraries are summarized in Table 2.6.

Table 2.6. Chlorodiiodomethane and bromodiiodomethane analytical parameters. Note that retention times of each analyte are based on analysis on column B in Table 2.3.

Compound	Primary m_1/z	Secondary m_1/z	Formula	MW [g mol^{-1}]	RT [min]
Chlorodiiodomethane	174.9	301.8	ClCHI$_2$	176.4	9.45
Bromodiiodomethane	218.8	345.8	BrCHI$_2$	267.8	11.55

2.4.6 Quality assurance and quality control

All analysis was performed within one week of sample collection and consisted of control blanks, method blanks, fiber blanks, ultrapure water blanks, and check standards. In addition, thermal degradation products of triiodomethane were evaluated at established method parameters by analyzing triiodomethane-spiked samples (400 µg/L triiodomethane). Signal responses of all blanks were established to be below MDLs and all check standards and recoveries were 100%±30% of the spiked concentration.

2.5 Microbial community analysis

Samples were collected in sterile 1 L amber bottles after 72 hr of BAF treatment. Approximately, 1 L of sample was filtered through Sterivex™ filters (Millipore Sigma, Burlington,MA). Genomic DNA was extracted using a DNeasy PowerLyzer PowerSoil DNA extraction Kit (Qiagen, Inc., Germantown, MD) as specified by the manufacturer. DNA preparation included a 45 second bead-beating step using a BioSpec Mini-Beadbeater-16 (BioSpec Products Inc. Bartlesville, OK).

DNA was quantified using a Qubit Fluorometer and a Qubit dsDNA High Sensitivity Assay Kit (Thermo-Fisher, Inc.). DNA samples were amplified using 515F (5'GTGCCAGCMGCCGCGGTAA3') and 806R (5'GGACTACHVGGGTWTCTAAT3') primers following the two-step amplification and barcoding strategy described elsewhere [53]. Illumina MiSeq sequencing targeting the V4 region of bacteria and archaea was performed by the Duke University Center for Genomic and Computational Biology using Illumina 2X250 chemistry. Post sequencing, data was demultiplexed using Sabre allowing for zero barcode mismatches (https://github.com/najoshi/sabre). The rRNA sequences (called 'amplicon sequence variants' or ASVs) [54] were initially analyzed using DADA2 [55] for the following: removal of PCR primer sequences and low-quality bases, merging paired end reads, taxonomy assignment using Silva Version 128 [56], and ASV table construction. Quantitative Insights into Microbial

Ecology (QIIME) version 1.9 was used to align and filter ASVs and construct a phylogenetic tree. The ASV table, taxonomy table, metadata, and phylogenetic tree were then imported into Phyloseq [57]. Prior to constructing heatmaps the ASV table was converted to compositional (i.e., relative percent) and filtered to retain ASVs representing > 0.1% of a samples' composition. The R packages Ampvis2 [58] and ggplot2 [59] were used to visualize the resultant heatmap.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Produced water quality

A summary of the influent and effluent water quality is provided in Table 3.1. Values for EC (average 31.4 mS/cm), pH (average 6.7), and total dissolved solids (average 16,700 mg/L) in BAF influent did not show significant fluctuations throughout the three sampling campaigns (Tables 3.1 and 3.2 (pg. 20)). Removal of TN and DOC were variable through the different time-stages of treatment with an average removal of 54±22% (min. 7-%/max. 79%) and 43±25% (min. 17%/max. 94%), respectively. TN removal was likely attributed to the biodegradation of organic nitrogen (not measured) and ammonia, which accounted for most of the nitrogen (24.8 mg/L NH_3-N) present in the PW water utilized in this study. Concentrations of nitrate and phosphate were below their respective MDLs in all samples. Dissolved iron concentrations in the BAF influent were relatively low, as expected from the oxidation pre-treatment of PW with $KMnO_4$. Iron and manganese concentrations show a decrease of iron during treatment and an increase of manganese, which indicate a complex environment where sorption and desorption mechanisms allow breakthrough of manganese and removal of iron. Breakthrough of inorganic constituents was also observed in some BAF columns, where iodide concentrations increased by 17% during treatment.

Table 3.1. General water quality parameters of DJ Basin PW. The first set of water quality parameters were measured at all stages of analysis (Stages I through III) while the second set of parameters were only analyzed at stage I. Values represent average BAF influent and effluent values and standard deviations.

Parameter	Unit	Influent	Effluent
# of samples (n)		3	27
pH	S.U.	6.7±0.0	7.2±0.2
Electrical Cond.	mS/cm	31.4±0.8	32.0±0.7
DOC	mg/L	420±141	192±95
DOC removal	%	-	54±22
Total nitrogen (TN)	mg/L	31±2	22±5
TN removal	%	-	43±25
Iodide (I^-)	mg/L	48±6	33±16
Iodide transformation	%	-	32±30
Sample size (n)		1	9
Bromide (Br^-)	mg/L	123	120.3±6.8

Table 3.1 Continued

Parameter	Unit	Influent	Effluent
# of samples (n)		1	9
Chloride (Cl$^-$)	mg/L	11347	10,576±354
Ammonia (NH$_3$-N)	mg/L	24.8	-
Nitrite (NO$_2^-$)	mg/L	19.1	17.8±1.4
Nitrite (NO$_2^-$-N)	mg/L	5.8	5.4±0.4
Nitrate (NO$_3^-$)	mg/L	BDL	BDL
Calcium (Ca^{2+})	mg/L	325	335±19.1
Magnesium (Mg^{2+})	mg/L	36	37.4±2.1
Iron (Fe)	mg/L	1.2	0.4±0.3
Manganese (Mn)	mg/L	3.9	4.2±1.4
Sulfur (S)	mg/L	9.2	10.3±0.7
Sodium (Na$^+$)	mg/L	4815	5,134±193

3.2 Halide concentrations

Iodide, chloride, and bromide were measured at concentrations substantially above those typical of surface water environments. Iodide concentration in the BAF influent ranged from 40.2 to 53.0 mg/L I$^-$ and in the BAF effluent it ranged from 5.3 to 62.2 mg/L I$^-$. Average bromide concentration in the BAF influent was 123 mg/L Br$^-$ and the concentration in the nine BAF effluent samples ranged from 105.9 to 131.5 mg/L Br$^-$. Chloride concentration in the BAF influent exceeded 11,000 mg/L Cl$^-$, and the concentration in the BAF effluents ranged from 9,626 to 10,811 mg/L Cl$^-$ (Table 3.2 (pg. 20)). Bromide and chloride concentrations did not change substantially (less than 15% variability) in any of the analyzed BAF effluent samples compared to the concentration measured in the influent sample. This may indicate that bromide and chloride were not readily transformed to other species (e.g., Br$_2$/Cl$_2$, HOBr/HOCl, etc.) or utilized within the system. Unlike bromide, iodide in PW changed substantially during treatment with the highest observed concentration decrease from 53.0 to 5.3 mg/L I$^-$, or a 90% decrease during treatment. A study by Oetjen et al. (2018) showed a linear increase of iodide, bromide, and chloride concentrations as an O&G production well transitioned from FB to PW period at which concentrations remained relatively constant during the PW period [6]. This suggests that a substantial portion of the iodide in PW originated from the rock formation (geogenic) rather than the fracking fluid injected (anthropogenic) to stimulate well production.

3.3 HS-SPME-GC-MS method validation and analysis of IOCs

Method validation was achieved with recovery experiments to quantify extraction performance for chloroiodomethane, diiodomethane, and triiodomethane and their deuterated analogues. Extraction efficiency tests were conducted at three different concentration levels (i.e., 25, 500, and 2500 µg/L) using the HS-SPME-GC-MS method developed in this study (Appendix A). Recoveries for this method were within 70–110% and standard deviations ranging from 1% to 14% (Figure 3.1). Additionally, calibrations achieved acceptable linearity for each analyte when calibration curves were adjusted above the MDLs ($R^2 > 0.989$). MDL and MQL were in the range of 4.4–19.2 µg/L and 10.5–53.2 µg/L, respectively, and were acceptable for this study.

Figure 3.1. Recovery efficiency of three selected volatile IOCs in BAF influent (solid bars; n=3) and effluent samples (dashed bars; n=4) at three different concentration levels.

In previous studies, diiodomethane and iodomethane were identified as thermal degradation products of triiodomethane [60]. Analysis of triiodomethane degradation products during degradation tests did not reveal formation of diiodomethane or iodomethane in

triiodomethane spiked samples (n=3, 400 µg/L triiodomethane) that were analyzed at established method and analytical conditions. Diiodomethane signal during this experiment was similar to the signal of the fiber blanks and were below MDL. The presence of iodomethane was only evaluated using selected ions, m/z 142, 127, and 141.

3.4 Emergence of IOCs during BAF treatment

Chloroiodomethane, diiodomethane and triiodomethane were measured at concentrations higher in the BAF effluent compared to the BAF influent. The highest concentration of triiodomethane measured was 4,316 µg/L in BAF 3 during stage I. The highest concentration of diiodomethane was 442 µg/L in BAF 8 during stage II (Table 3.2). Chloroiodomethane was not detected above the MDL of 6 µg/L in any of the BAF column effluents, except for BAF 8 at treatment stage II, where its concentration was measured to be 16.6 µg/L. The concentration of chloroiodomethane is likely underestimated due to the high volatility of this compound in an open system. The augmentation of IOCs was observed through nine BAF columns operated at different conditions. Additionally, iodide utilization within the system was also observed. The observed iodide concentrations can be due to sorption/desorption mechanisms within the system, utilization by iodide accumulating and oxidizing bacteria, and transformation to other iodide species within the system. Examination of other prominent peaks in total ion chromatograms did not reveal the presence of other halogenated DBPs in samples analyzed immediately after BAF treatment (Figure 2.2 (pg. 12)).

It is worth noting the exceptionally high triiodomethane concentration measured in BAF 3 effluent compared to all other BAFs during stage I of sampling. Analysis of iodide concentrations in BAF 3 showed that approximately 48 mg/L (90%) of iodide transformed into other iodide species. After the high concentration of triiodomethane measured, the overall IOC concentration declined substantially over the next two sampling stages. System performance was not affected after sampling stage I with 93% and 70% DOC removal in stage II and III respectively. BAF 3 was a control column (no nutrients were added) filled with spent GAC that had been previously used for biological treatment of surface water, and achieved relatively high DOC removal throughout the three stages of sampling (Table 3.2). BAF 3 also achieved higher TN removal and bromide transformation/removal, which points to a more biologically and chemically dynamic system in comparison to the other BAF columns.

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Table 3.2. Concentration of three iodinated organic compounds and several water quality parameters - iodide, total nitrogen (TN), dissolved organic matter (DOC), pH, and EC measured in raw produced water and after 72 hours of biological treatment at three treatment stages (I: week 4; II: week 8; and III: week 13 after starting the system (week 1)).

		BAF Column									
		$1^{S,1}$	$2^{S,2}$	$3^{S,3}$	$4^{N,1}$	$5^{N,2}$	$6^{N,3}$	$7^{D,1}$	$8^{D,2}$	$9^{D,3}$	in
	Unit	Stage I									
CH_2ClI	µg/L	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
CH_2I	µg/L	7.2	BDL	14.7	10.2	6.7	BDL	10.3	37.2	4.6	21.5
CHI_3	µg/L	50	248	4316	236	253	265	90	90	290	101
DOC	mg/L	212	182	101	209	259	349	300	283	385	616
TN	mg/L	18	13	7	20	18	19	19	19	22	33
Iodide	mg/L	47	62	5	46	46	33	52	56.6	43	53
Bromide	mg/L	121	115	106	127	123	119	121	132	119	123
Chloride	mg/L	10414	10632	9626	10755	10761	10811	10783	10700	10706	11337
Iron	mg/L	0.8	0.3	0.1	BDL	BDL	BDL	0.3	0.6	BDL	1.3
Manganese	mg/L	6.3	7.0	2.3	3.9	3.5	4.0	4.0	3.4	3.3	3.9
pH	S.U.	7.3	6.9	7.0	7.3	7.0	7.6	6.9	7.1	7.4	6.6
EC	mS/cm	32.8	32.5	32.7	33.2	33.0	32.6	32.4	32.6	33.3	32.1
		Stage II									
CH_2ClI	µg/L	BDL	BDL	BDL	BDL	BDL	BDL	BDL	16.6	BDL	BDL
CH_2I	µg/L	14.4	8.1	6.4	9.3	5.4	43.5	15.2	442	BDL	BDL
CHI_3	µg/L	-	688	99	1341	306	905	246	24	408	BDL
DOC	mg/L	34	18	19	116	165	183	123	177	225	292
TN	mg/L	18	16	19	28	29	29	27	26	28	31
Iodide	mg/L	24	12	40	20	35	20	39	10	34	40
pH	S.U.	7.3	7.4	6.9	7.5	7.5	7.4	7.2	7.4	7.3	6.7
EC	mS/cm	31.1	31.8	31.7	31.2	31.8	31.8	31.4	31.9	31.1	31.1
		Stage III									
CH_2ClI	µg/L	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
CH_2I	µg/L	9.6	9.3	9.7	134	29.2	15.1	19.8	19.7	8.7	BDL
CHI_3	µg/L	425	247	272	1411	791	946	949	361	582	57
DOC	mg/L	89	111	107	211	262	239	263	268	293	351
TN	mg/L	19	19	20	24	25	25	25	26	24	28
Iodide	mg/L	40	50	50	25	16	20	17	6	43	52
pH	S.U.	7.2	6.7	7.1	7.1	6.9	6.9	7.4	7.4	7.4	6.7
EC	mS/cm	30.7	31.3	31.7	31.6	30.9	31.8	32.3	32.3	32.0	30.6

S: Spent media, N:Norit media, D: Darco media, 1: nutrient solution 1, 2: nutrient solution 2, 3: control, BDL: below detection limits, CH_2ClI: chloroiodomethane, CH_2I_2: diiodomethane, CHI_3: triiodomethane, $CHClI_2$: chlorodiiodomethane.

3.5 Emergence and fate of IOCs after prolonged storage of BAF effluent

Samples collected in stage III were re-evaluated for IOC re-occurrence after prolonged storage (137 days). Concentrations of IOCs for four sets of samples are summarized in Table 3.3. For all columns, there was a substantial increase in IOC concentrations from the initial sampling campaign as compared to those evaluated after 137 days of storage. Diiodomethane, which was

only measured at a maximum concentration of 442 µg/L at stage II (BAF 8), was measured at concentrations exceeding 2500 µg/L in 6 samples analyzed. Chloroiodomethane was also measured above the MQL in all samples with a maximum concentration of 306 µg/L. This specific compound was not detected above the MDL during the initial analysis during stage III. In this set of samples, BAF 8 was the only effluent sample where triiodomethane was detected below the limits of detection whereas all other effluent samples it was measured at concentrations exceeding 2500 µg/L. The two semi-quantified samples (chlorodiiodomethane and bromodiiodomethane) were also measured at concentrations above the MDLs (based on diiodomethane MDLs), where chlorodiiodomethane was observed at relatively higher abundance than bromodiiodomethane—concentrations above 2500 µg/L and 563 µg/L were estimated for chlorodiiodomethane and bromodiiodomethane, respectively.

Table 3.3. Evaluation of the fate of IOCs from stage III samples after 137 day of storage. Samples with exceeding concentrations of 2500 µg/L were only calculated measured based on the highest calibration standard.

		BAF Column									
		$1^{S,1}$	$2^{S,2}$	$3^{S,3}$	$4^{N,1}$	$5^{N,2}$	$6^{N,3}$	$7^{D,1}$	$8^{D,2}$	$9^{D,3}$	in
	Unit	Stage III (137 days storage)									
CH_2ClI	µg/L	21.4	17.0	42.7	132	84.2	33.0	124	306	131	BDL
CH_2I_2	µg/L	895	594	>2500	>2500	>2500	1918	>2500	>2500	>2500	BDL
CHI_3	µg/L	>2500	>2500	>2500	>2500	>2500	>2500	>2500	BDL	>2500	BDL
$CHClI_2$	µg/L	>2500	>2500	>2500	>2500	2021	>2500	1157	30.2	>2500	BDL
$CHBrI_2$	µg/L	284	541	563	153	100	337	46.6	BDL	364	BDL
DOC	mg/L	90.5	112	105	207	246	237	244	234	283	340
TN	mg/L	20.7	20.3	22.2	28.0	28.1	27.9	28.1	27.9	29.7	35.1

S: Spent media, N:Norit media, D: Darco media, 1: nutrient solution 1, 2: nutrient solution 2, 3: Control, BDL: below detection limits, CH_2ClI: chloroiodomethane, CH_2I_2 :diiodomethane, CHI_3: triiodomethane, $CHClI_2$: chlorodiiodomethane, $CHBrI_2$: bromodiiodomethane.

3.6 Distribution of IOCs in BAF effluent samples

Evaluation of the relative abundance of each compound at each treatment stage revealed that triiodomethane is predominantly found in most effluent samples (at least 90% contribution of the total IOCs measured in 21 samples (n=21) analyzed in stage I, II, and III) (Figure 3.2). BAF 8 showed a different pattern of IOC distribution compared to the other BAF samples analyzed during the same sampling campaign. During stage II, BAF 8 predominantly contained diiodomethane followed by chloroiodomethane, and during stage III (137 days) it predominantly

contained diiodomethane while triiodomethane was below detection limits (Figure 3.2 and Table 3.3 (pg. 21)). This was not expected based on the high concentrations of triiodomethane measured in all other BAF effluent samples during the initial sampling stages. Diiodomethane has been observed to form from biological activities of several microorganisms [61]. This is a potential indication that this specific BAF effluent sample is biologically active with microorganisms with preferential diiodomethane formation.
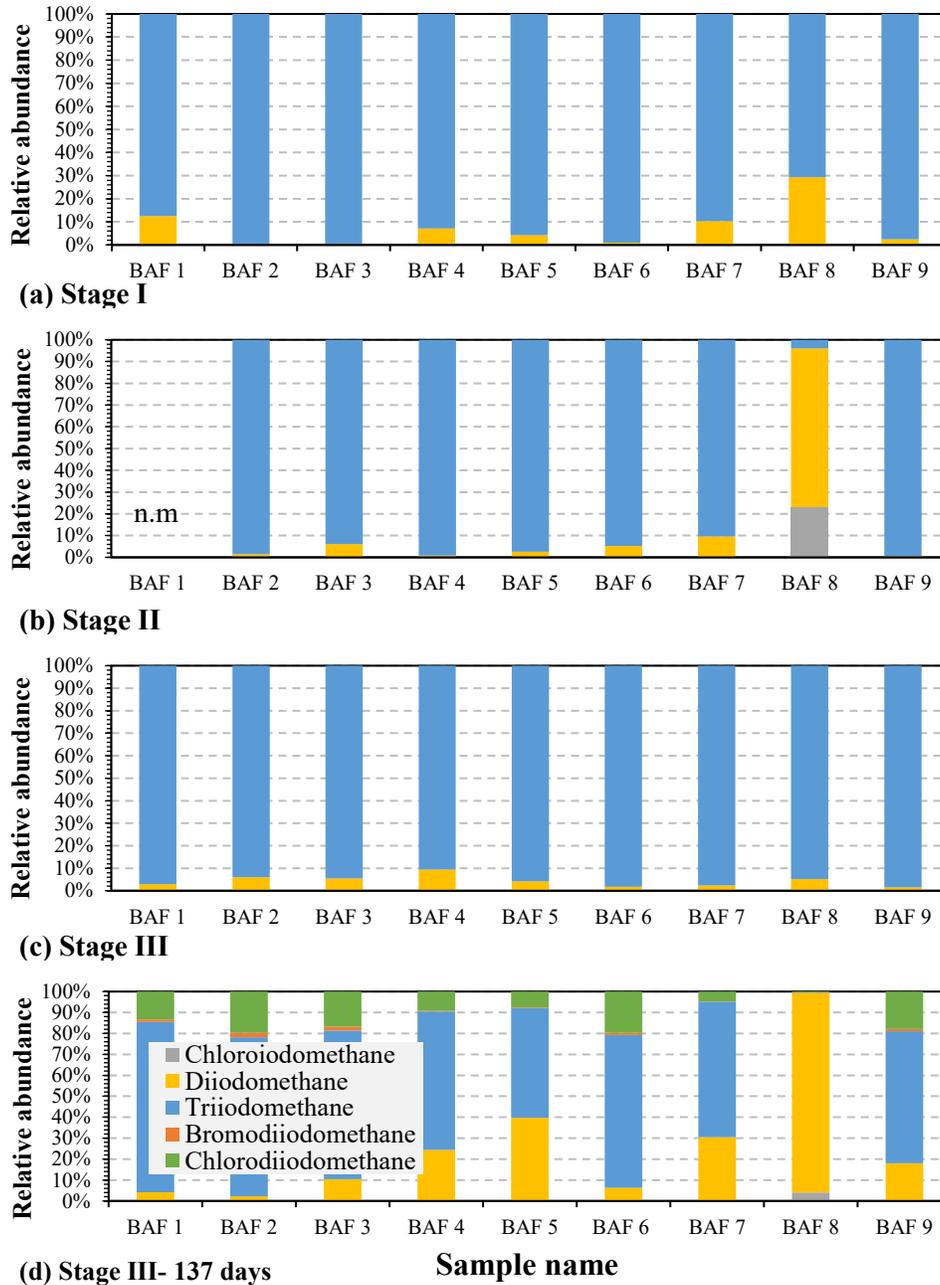

Figure 3.2. Estimated contribution of five IOCs in nine samples of biologically treated produced water during (a) stage I, (b) stage II, (c) stage (III), and (d) stage III after 137 days of storage.

In samples analyzed after prolonged storage (Figure 3.2d (pg. 22)), IOC distribution was mostly dominated by triiodomethane, diiodomethane and chlorodiiodomethane. Chlorodiiodomethane was not detected in BAF samples during initial sampling but was semi-quantified at significant concentrations after prolonged storage. BAFs with the same spent GAC media (BAF 1-3) showed similar IOC distribution compared to those with the Darco® 12×40 GAC (BAF 7-9), which showed unusually high abundance of diiodomethane in BAF 8 as well as less bromodiiodomethane and chlorodiiodomethane in BAF 7. Additionally, BAF with no nutrient solution added (BAF 3, 6, and 9) showed similar IOC distributions compared to those with nutrient solution 1 or 2 added during treatment.

3.7   Microbial community analysis

Microbial communities in PW before and after biological treatment were evaluated to understand the role of IOB in the formation of IOCs.

3.7.1 Iodide oxidizing bacteria in BAF effluent

The presence of IOB in O&G produced water was previously reported in the literature [62]. Studies conducted in BAFs used in O&G produced water treatment have conducted microbial community analysis of granular activated carbon in BAFs, but have not reported microbial community analysis of the treated wastewater [11, 63]. Because GAC and the treated water provide different micro-niches for microbial development, microbial community analysis of biologically treated water was analyzed in efforts to identify IOB and other microbial communities. 16S rDNA analysis of four streams treated independently through a BAF system revealed the presence of organisms of the *Roseovarius* and *Iodidimonas* genus (Figure 3.3), which form part of the genus of IOB [27, 62, 64]. Interestingly, organisms of the *Roseovarius* and *Iodidimonas* genus were found at 0.2% and 0%, respectively, in untreated produced water. In comparison, in produced water treated through BAFs the relative abundance was substantially higher (51.5% and 5.9%, respectively).
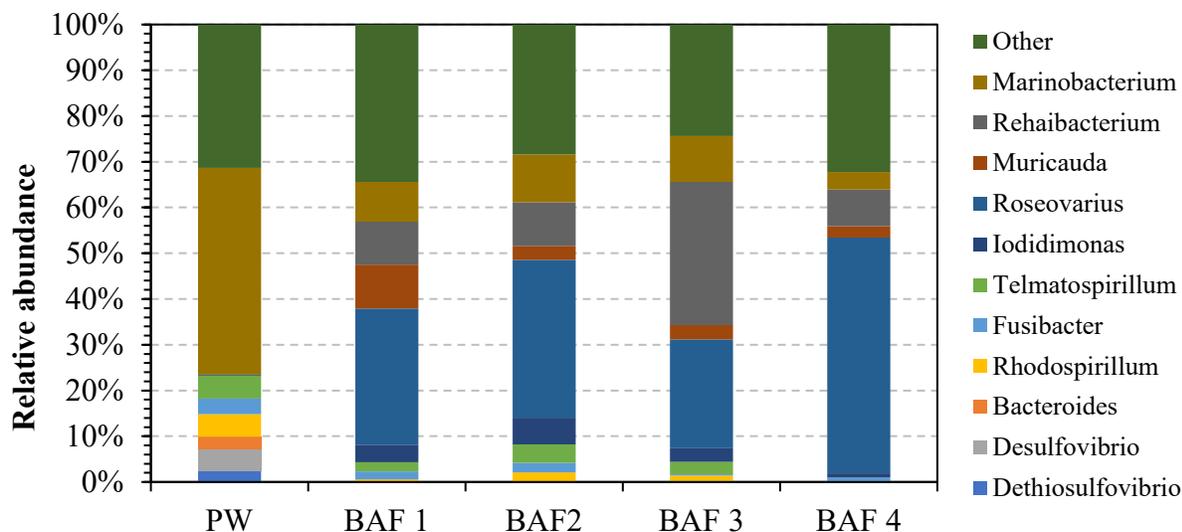
Figure 3.3. Relative abundance of microorganisms in the effluent of the best performing BAF columns. Extracted DNA sequences were ranked based on genus taxonomy.

The formation of halogenated organic compounds mediated by biological iodide oxidation has been observed in previous studies [27, 30, 65, 66]. Two compounds analyzed in this study, diiodomethane and chloroiodomethane, are also known to form biotically [28, 38, 65, 66] by microorganisms such as *Bjerkandera Adusta* (Fungi) [61] and multiple *α-proteobacteria* strains [27]. Also, Cyanobacteria have been previously found [28] in ponds with high release rates of diiodomethane. Analysis of biologically treated PW in this study and of microorganisms in GAC of BAF columns operated under similar conditions and similar PW stream [11] did not indicate the presence of cyanobacteria; yet, cyanobacteria have been previously reported in a biological treatment system used to treat O&G produced water and a sequencing batch reactor-membrane bioreactor co-treating residential and produced water from the DJ basin [13, 14]. Amachi et al. [27] presented and isolated several IOB responsible for extracellular enzymatic iodide oxidation to iodine. Organic iodine species (diiodomethane and chloroiodomethane) in cultivations with IOB strains were also found at concentrations higher than iodine. The formation of organic iodine was determined to originate from reactions of biologically mediated reactive iodine species with organic matter. As postulated by Amachi et al., iodide oxidation to reactive iodine species is likely mediated by an extracellular oxidase enzyme [27]. Given the nature of IOB, these organisms are likely tolerant to high levels of halogens, iodide and iodine and readily produce I_2 due to its toxicity and oxidative reactivity that can function as a bactericidal. Based on DNA results IOB are present in iodide-rich PW brines at very low abundances because PW

brines are in contact with ancient marine sediments rich in halogens. Additionally, IOB have been previously found in O&G wastewater impoundments [62] and natural gas brines [64], but are not ubiquitous unless specific growth conditions are developed in iodide rich wastewater.

3.7.2 Microbial communities in BAF effluent

Evaluation by family taxonomy (Figure 3.4) showed a substantial shift in distribution of microbial communities. There was a substantial increase in *Proteobacteria* of the *Rhodobacteraceae* family and a decrease of *Oceanospirillaceae* after treatment. Organisms belonging to the *Rhodobacteraceae* family can be chemotrophic and phototrophic with diverse metabolisms including facultative aerobes and anaerobes [67, 68]. *Oceanospirillaceae* include organisms that are halophilic and aerobic and mainly originate from marine environments [69]. Some species of this family can degrade petroleum derived compounds [69] and their presence in produced water is explained by high salt concentrations in this waste stream that is in contact with ancient marine shale formations.

Figure 3.4. Relative abundance of microorganisms in four biologically treated produced water streams. Extracted DNA sequences were ranked based on family taxonomy.

Besides the emergence of organisms of the *Roseovarius* and *Iodidimonas* genus, organisms associated with *Muricauda* and *Rehaibacterium* genus were also augmented during biological treatment from 0.0% in PW to a maximum of 9.6% in BAF treated water and 0.0% to a maximum of 31.1%, respectively. *Muricauda* belong to a group of the family

Flavobaacteriacea and species in this genus and family (e.g., *Muricauda ruestringensis gen. nov., sp. nov.)* have been classified as a non-motile, facultative anaerobes associated with marine environments [70]. As a marine bacterium, the organism described by Bruns et al. [70] can tolerate salt concentrations similar to that of seawater. In contrast, *Rehaibacterium* of the *Xanthomonadaceae* (Figure 3.4) family have been described as thermotolerant, strictly aerobic, with optimal growth at 0-4% NaCl (w/v) [71].

3.8   Evaluation of biotransformation pathways

Evaluation of transformation pathways was conducted by examination of correlations between water quality parameters (e.g., DOC and iodide concentration) and IOC concentrations for columns with the same GAC (n=9) and nutrient type (n=9) to determine if GAC or nutrient type played a role in IOC formation. BAFs with the same nutrient type had a stronger correlation between iodide concentration and total IOC concentration (r=0.75–0.83) in comparison to BAFs with the same GAC (r=0.59–0.74). Unlike, other studies [72], DOC concentrations did not strongly correlate with IOC or triiodomethane concentrations. Only BAFs with Darco® 12x40 GAC and nutrient 1 had a moderately strong negative correlations between DOC removal and total IOC concentration (r=0.69 and 0.59, respectively; while all others had r<0.31). Iodide and IOC concentrations varied substantially throughout the 3 sampling stages (Table 3.2 (pg. 20)) for each individual column, which supports the unpredictability of IOC formation in a BAF system because of the complexity of the wastewater treated and the dynamics of each individual BAF.

An overview of the distribution (evaluated by the location of the minimum and maximum) and variability (evaluated by the interquartile range) of total IOC formation, iodide removal, and DOC removal in columns with the same GAC, same nutrient solutions, and same sampling stages is provided in Figure 3.5 (pg. 28). BAFs with spent GAC and Darco® GAC exhibited a similar distribution of IOC concentrations with the exception of an outlier in the spent GAC data set (BAF 3 stage I) (Figure 3.5c(i)). There was not a distinct pattern of distribution of iodide removal for BAFs with any GAC; yet, BAFs with Norit® GAC had a median significantly higher than the other GAC medias (Figure 3.5b(i)). DOC removal was substantially higher for BAFs with spent GAC, with more than 70% removal of DOC observed for at least half of the data set (Figures 3.5a(i)). The addition of nutrient solutions did not seem to affect IOC concentrations (Figure 3.5c(ii)). The highest and lowest IOC variability and

distribution was observed for BAFs with nutrient solutions 1 and 2, respectively. The distributions of iodide removal were not substantially different for BAFs with different nutrient solutions (Figure 3.5a(ii)) . Evaluation over three sampling stages showed an increase in IOC concentrations (Figure 3.5c(iii) over time with a similar variability during stages II and III. Iodide concentrations showed a similar increase in average concentration and distribution as IOC concentration (Figure 3.5b(iii)). The observed increase in IOC concentrations over the 3 sampling stages in conjunction with increasing iodide removal further supports that iodide introduced to the system in produced water is likely utilized/transformed within the system for reactions leading to IOC formation.

Based on biological data, there is an indication of a rapid transition (72 hours) of microbial communities present in PW during biological treatment using BAFs. The presence of iodide-oxidizing genus further reinforces the formation of IOCs and I-DBPs during treatment as a biogenic byproduct of treatment. Although many studies have not directly identified IOB as dominant species in PW, they comprise a small fraction (0.2%) of organisms classified in the α-proteobacteria phylum and seem to develop in PW during treatment. Additionally, the small communities of IOB (<0.2%) present in PW seem to develop during treatment and might be responsible for iodination reactions. In addition to the microbial analysis, two additional observations that support IOB as the iodide selective oxidizing mechanisms that contributes to the formation of I-DBPs and IOCs in the BAFs include the presence of two halogenated organic compounds (diiodomethane and triiodomethane) with only iodide moieties formed at high concentrations and the significant change of iodide during the initial sampling stage when iodide was transformed by 90% and triiodomethane was measured up to 4,316 µg/L (BAF 3). Also, the implementation of full scan mode did not reveal the presence of other halogenated DBPs. In addition, the presence of two compounds of biogenic origin (CH_2ClI and CH_2I_2) further reinforce biogenic influence on IOC formation in BAFs treating PW.

Figure 3.5. Evaluation of (a) system performance (DOC) and (b) iodide transformation on (c) IOC concentration for BAFs with the same GAC media, nutrient solution and sampling time. Note that each data set is composed of nine (n=9) samples analyzed for a given GAC, nutrient solution or sampling stage. DOC and iodide removal/transformation were evaluated based on the ratio of effluent concentration (C) to influent concentration (C_o).

The presence of IOCs and IOBs in O&G PW treated through BAFs reinforces the idea of a biogenic influence in the formation of IOCs and I-DBPs. As previously mentioned, iodide is readily bioavailable in the form of iodide (I^-) which has been reported at concentrations up to 53 mg/L I^- in this study and up to 53.5 mg/L I^- in other studies [34]. IOB likely oxidize iodide using a extracellular oxidase transforming iodide to reactive iodine species such as I_2 [27]. Organic matter utilization by bacterial consortia developed within the GAC and treated water, transform complex organic matter into organic matter that can readily interact with reactive iodine species initiating halogenation of organic matter into IOC and I-DBPs. I-DBPs in this study likely formed through haloform reaction of methyl ketones and other organic matter. Studies have suggested that the nature of organic matter can affect the formation of different DBPs [44, 72, 73]. The I-DBPs mentioned in this study are part of a sub-category of disinfection byproducts that have been previously studied to identify organic precursors. These precursors include metadihydroxybenzene phenolic structures, β-diketones, and β-ketoacids [43, 44]. The distinct media and nutrient type in each column most likely lead to the development of distinct microbial communities with specific organic substrate utilization and formation of different organic subunits. This would cause a divergence in quality and nature of organic matter that would result in the formation of variable concentrations of IOCs including triiodomethane, diiodomethane, and chloroiodomethane.

3.9   Implications for treatment, discharge, reuse and DBP formation

Although the I-DBPs (triiodomethane, bromodiiodomethane, and chlorodiiodomethane) investigated in this study, or any other iodinated I-DBPs, are not regulated by the EPA, four other similar DBPs (chloroform, bromodichloromethane, dibromochloromethane, and bromoform) are regulated to a total concentration of 80 µg/L in drinking water by the United States Environmental Protection Agency [40, 74]. The potential formation of regulated and non-regulated DBPs at concentrations that exceed the established regulatory concentrations could potentially hinder beneficial reuse of reclaimed O&G wastewater.

From an air quality perspective, organic iodine has been shown to form iodine radicals that readily react with atmospheric ozone leading to ozone depletion in areas with high release rates of organic iodine (e.g., marine microbiota) [29, 65, 75]. Global atmospheric conditions will

be affected by organic iodine species as well as the global iodine budget and cycling. The release of halides that were previously sequestered in ancient marine sediments can change current iodine and halide global budgets, increasing the occurrence of iodinated and halogenated precursors in the vulnerable environments and the atmosphere.

Additionally, formation of IOC and I-DBPs that are potentially mediated by biological reactions, require re-evaluation of management strategies of O&G derived waste streams that could amplify IOB and their proliferation in other surface environments during reuse. This can be problematic because naturally occurring microbial communities in surface environments could be vulnerable to high concentrations of IOCs and reactive iodine species mediated by IOB.

Iodide is an important element for many pharmaceutical and commercial applications. Recovery of iodide from PW can provide additional economic advantages to reclaim and treat PW to minimize its impacts on the environment. Ion exchange resins can be a feasible pre-treatment alternative to recover iodide from PW. Yet, the presence of other extractable constituents like chloride can potentially hinder recovery strategies of halogens.

CHAPTER 4

CONCLUSION

In this study, we observed that IOCs form during biological treatment of produced at levels in the mg/L range. There were indications that the high levels of iodide introduced to the system in produced water were utilized within the system for halogenation reactions with organic matter. The three I-DPBs formed in the treated water are not commonly formed during water disinfection because iodide is not ubiquitous in surface streams and iodide-based disinfectants are not typically used for disinfection of drinking water. This study shows that there is/are factor(s) that affect the preferential formation pathway specific IOCs based on the observed distribution of IOCs at different stages of treatment and in BAFs operated at different conditions. Understanding preferential formation pathways for IOC and I-DBPs can greatly enhance our knowledge in DBPs for future mitigation strategies during drinking water disinfection. Studies in complex streams can help develop more data in regards to potential bond dissociation energies of organic matter and reactive halide species as well as region-selectivity and free energies of activation for competing reactions forming different I-DBPs and organic iodine species.

This research provided valuable information about the type and concentrations of IOCs formed during biological treatment of produced water. Although this research showed that IOCs are most likely formed by IOB more research is required to:

1) Evaluate what chemical and physical conditions allow IOB to develop so rapidly in BAF filters

2) Understand what type of organic matter readily react with iodine to form specific IOCs.

3) Evaluate the mechanisms utilized by IOB to oxidize iodide.

4) Assess the impact that IOB and reactive iodine on other microbial communities have in the GAC and the water

Monitoring microbial communities and IOCs in BAF's GAC and treated water can provide the answer as to what microbial communities are responsible for IOC. Additionally, analyzing BAF operating conditions such as dissolved oxygen, reduction potential as well as organic matter characterization can provide more answers in regards to the potential formation pathways and mechanisms.

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APPENDIX A

SPME METHOD DEVELOPMENT

Four extraction parameters were evaluated for solid-phase microextraction method developed for the analysis of IOCs. These extraction parameters were evaluated for extraction efficiency evaluated based on area counts and reproducibility evaluated based on standard deviation.
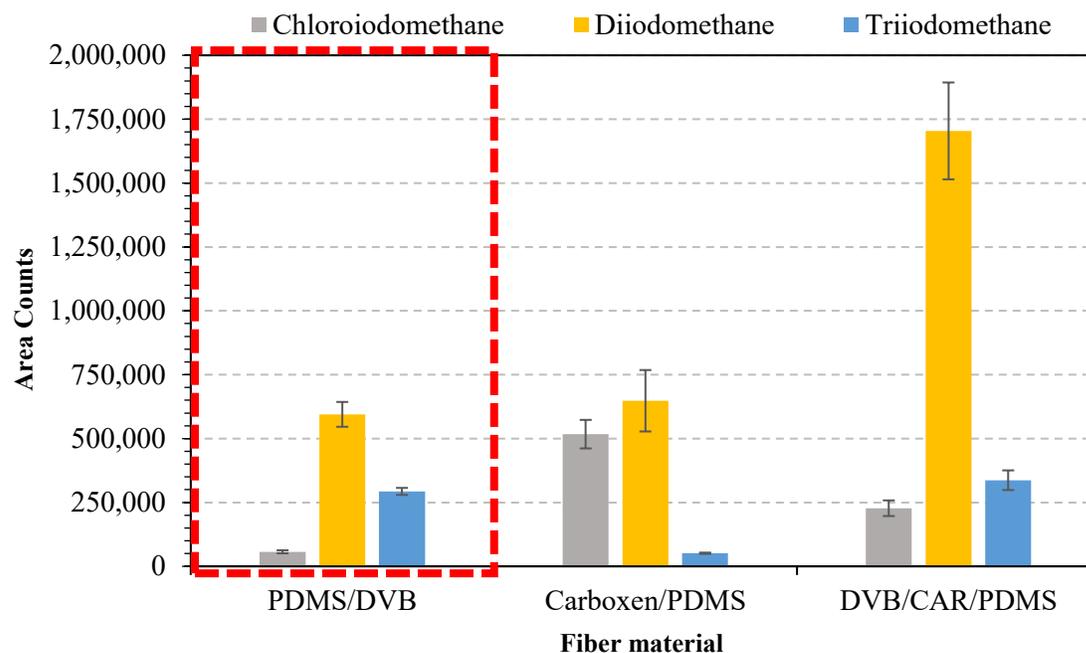

Figure A.1. Evaluation of fiber selection by comparison of extraction efficiency (area counts) vs. fiber material for three selected fibers. Analysis was performed on triplicate samples (n=3) spiked with 400 µg/L of each analyte. Reproducibility (standard deviation) and peak tailing were also considered for fiber selection. A double-phase PDMS/DVB dashed section) fiber was selected because minimal peak tailing and lower standard deviations were observed.

Figure A.2. Evaluation of extraction temperature by comparison of extraction efficiency (area counts) vs. extraction temperature at three extraction temperatures. Analysis was performed on triplicate samples (n=3) spiked with 400 µg/L of each analyte. An extraction temperature of 45ºC (dashed section) was selected based on efficient extraction of all compounds.

Figure A.3. Evaluation of extraction time by comparison of extraction efficiency (area counts) vs. extraction time. Analysis was performed on triplicate samples (n=3) spiked with 400 µg/L of each analyte extraction time of 60 minutes (dashed section) was selected because chloroiodomethane and diiodomethane had reach equilibrium and longer extraction times would significantly increase sample analysis time decreasing method applicability.

Figure A.4. Desorption time evaluation as represented by (a) extraction efficiency (area counts) vs. desorption time and (b) carryover potential. Analysis was performed on triplicate samples (n=3) spiked with 400 µg/L of each analyte. A desorption time of 2-3 minutes were found to be efficient. For this study a desorption time of 3 minutes (dashed section) was selected for the SPME method. A total desorption time of 5 minutes was employed by pre- and post-desorbing the fiber for 1 minute for each sample analyzed.

CORRECTION FACTORS AND MATRIX INTERFERANCES

Calibrations were carried out in both ultrapure water and influent BAF matrix. Both calibrations were confirmed to be linear ($R^2 \geq 0.989$) and were plotted against each other to yield a linear relationship (Table B.1). Matrix interferences were found to be concentration dependent and were adjusted according to Equation B.1.

$$S_1 = S_2\, m + b \qquad\qquad\qquad \text{B.1}$$

where S_1 represents the true quantification value of a sample, S_2 represents the estimated quantification of a sample using a calibration curve free of matrix interference (water-based calibration) and corrected with correction factor (m and b) measured in matrix-based calibration curves. For triiodomethane correction factors were only established in the high range calibration range (i.e., above the MQL of 53.2 µg/L).

Table B.1. Matrix correction factors for target analytes quantified using water and PW matrix calibration curves (n=5 for each range). Quantification was achieved by adjusting calculated concentrations with water-based calibration curves.

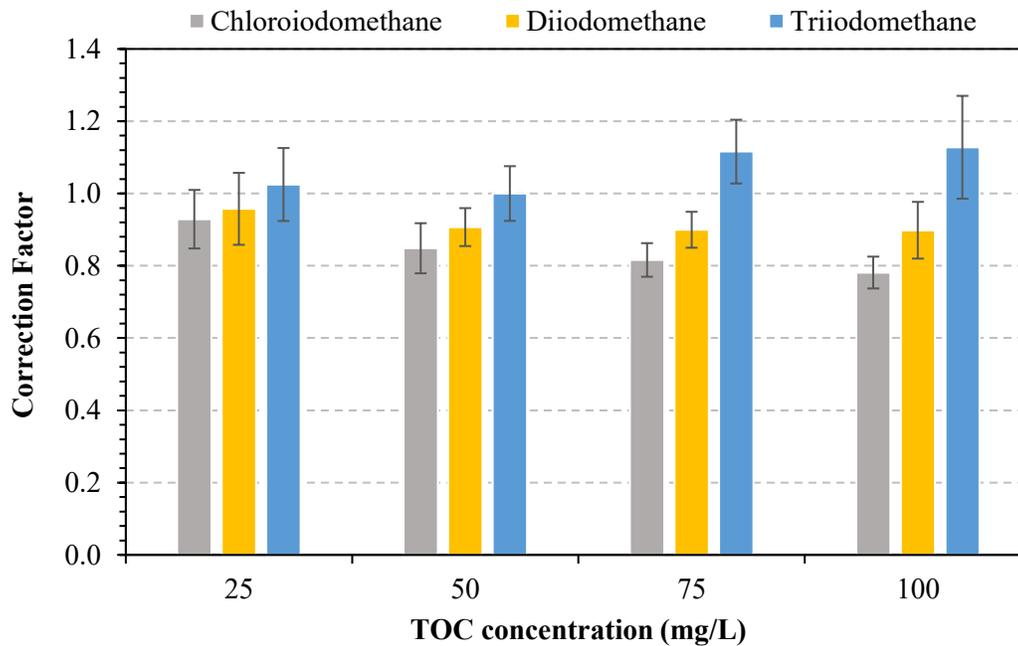| Compound	Low range calibration (1 µg/L to 100 µg/L)			High range calibration (50 µg/L to 2500 µg/L)		
	Slope (m) (µg/L H_2O • µg/L PW)	y-intercept (b) (µg/L PW)	R^2	Slope (m) (µg/L H_2O • µg/L PW)	y-intercept (b) (µg/L PW)	R^2
Chloroiodomethane	0.941	0.028	0.998	1.13	-1.93	0.990
Diiodomethane	0.953	0.002	1.000	0.96	-2.01	0.989
Triiodomethane	n.a.	n.a.	n.a.	0.81	-0.73	0.993

Figure B.1. Evaluation of matrix effects based on correction factors at different TOC concentrations. Samples were spiked with 400 µg/L of each analyte and extracted using method parameters.
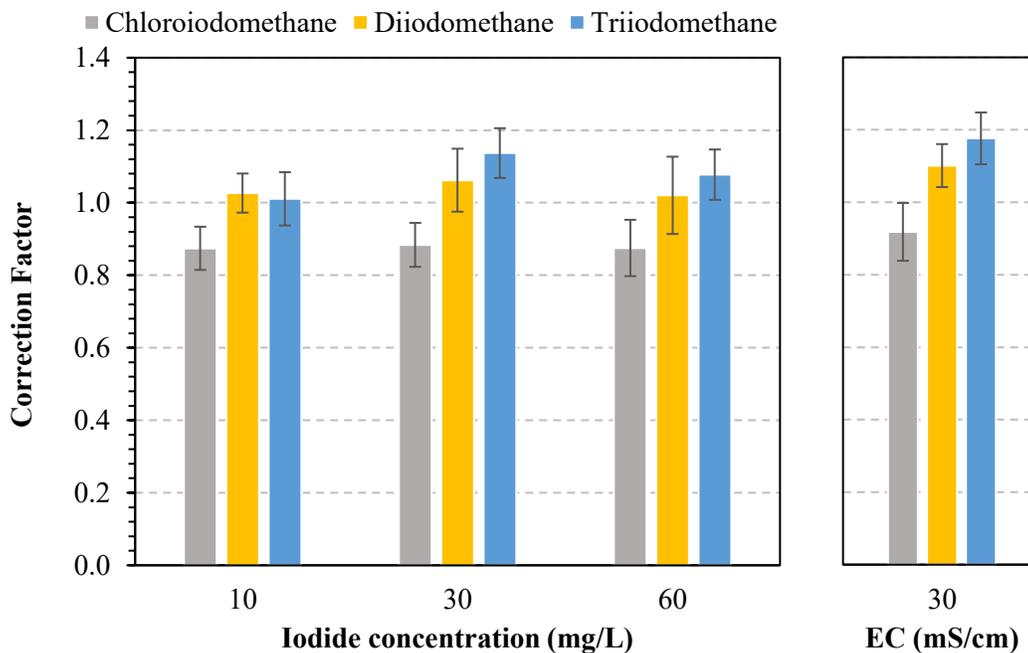

Figure B.2. Evaluation of matrix effects based on correction factors at different iodide concentrations and at the EC of produced water. Samples were spiked with 400 µg/L of each analyte and extracted using method parameters.

APPENDIX C

IDENTIFICATION AND SEMI-QUANTIFICATION

Additionally, implementation of full scan mode allowed for the identification of two additional unregulated I-DBPs—chlorodiiodomethane and bromodiiodomethane—in BAF effluent that had been stored for an extended period of time. Samples were collected and analyzed at treatment stage III and were thereafter stored in the refrigerator at 4 °C with no preservative addition and approximately 5 mL headspace. Analysis of all nine BAF columns, BAF influent and control samples was carried out in a similar manner as the initial analysis to ensure consistency.

In Table C.1, library searches for two prominent peaks detected in BAF samples are evaluated based on three factors in NIST library searches. Identification was conducted based on NIST library searches based on SI, RSI and probability values. SI indicates the direct match factor for the library spectrum and unknown sample, RSI is the reverse match for the library spectrum and unknown sample ignoring peaks in the unknown sample that are not in the library spectrum, and the probability is estimated based on the difference between hits in a given hit list [76]. An SI or RSI value of 999 represents a perfect match between the unknown sample and the library spectrum whereas values ranging from 900 to 999 are excellent matches, 800-900 are good matches, 700-800 are fair matches, and values less than 600 are poor matches. Abundance of interfering peaks (low abundance of unknown as compared to background noise) can yield poor matches. The mass spectra of peaks integrated at the same retention time in all nine BAF samples had excellent and good match factors for both SI and RSI values.

Table C.1. NIST Library search information for two I-DBPs identified in biologically treated PW. For chlorodiiodomethane, library hits of the 2nd most probable compound corresponding to the extracted mass spectra only had 0.95% match probability with $C_4H_6BrN_3$ (536 SI, 733 RSI). Similarly, bromodiiodomethane had a 2nd hit with 0.97% match probability with $C_9H_{11}Cl_2NO$ (528 SI and 661 RSI).

Sample name	Chlorodiiodomethane				Bromodiiodomethane			
	SI	RSI	Prob. (%)	S/N ratio	SI	RSI	Prob. (%)	S/N ratio
BAF 1	919	920	98.2	126,294	896	896	98.1	15,134
BAF 2	927	927	98.5	123,700	909	909	97.4	60,227
BAF 3	935	936	98.0	62,032	896	896	97.6	18,087
BAF 4	933	933	98.6	5,007	868	868	97.3	20,212
BAF 5	910	911	97.8	45,323	867	867	98.1	697
BAF 6	937	938	98.5	141,280	918	918	98.3	19,544
BAF 6 dup	922	923	98.3	122,537	882	882	97.5	61,722
BAF 7	923	924	98.7	25,514	894	895	97.1	4,027
BAF 8	820	822	98.6	1,242	n.a.	n.a.	n.a.	21.4
BAF 9	922	923	98.5	28,683	899	899	98.3	3,940
BAF influent	n.a.	n.a.	n.a.	6,377	n.a.	n.a.	n.a.	233.5

*n.a.-not applicable;

APPENDIX D

COMPOUND MASS SPECTRA

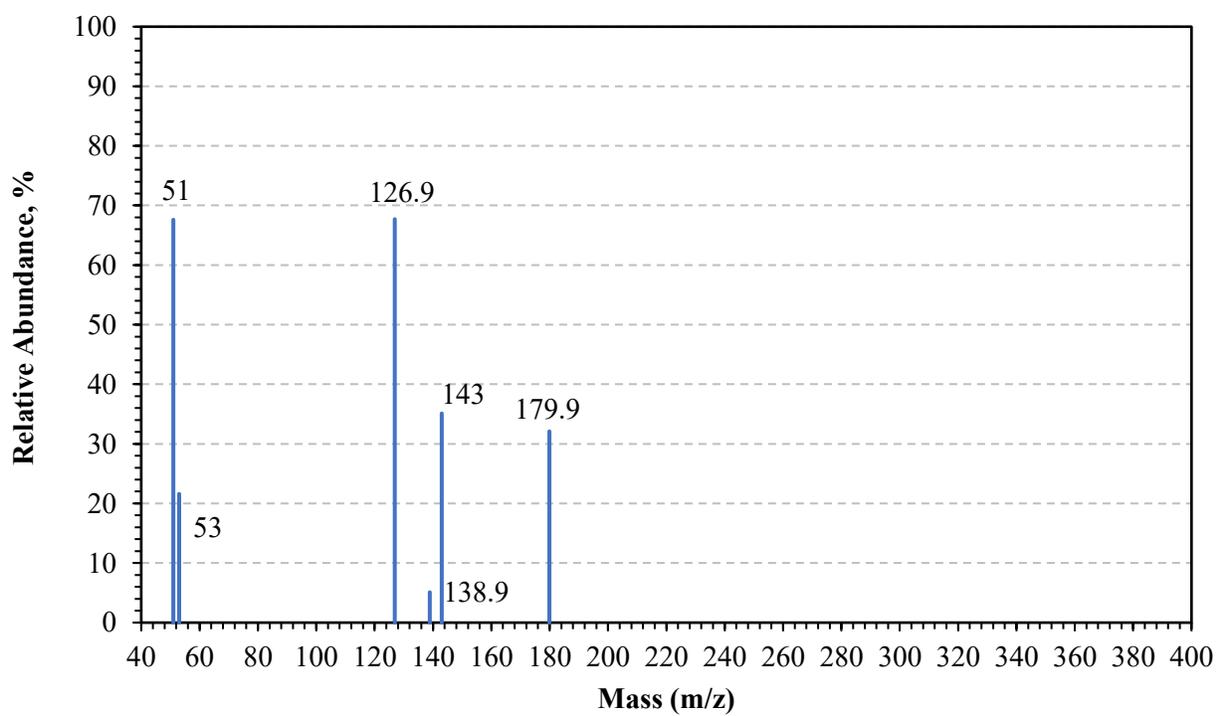Figure D.1. Mass spectra of chloroiodomethane from analytical standards and BAF samples.

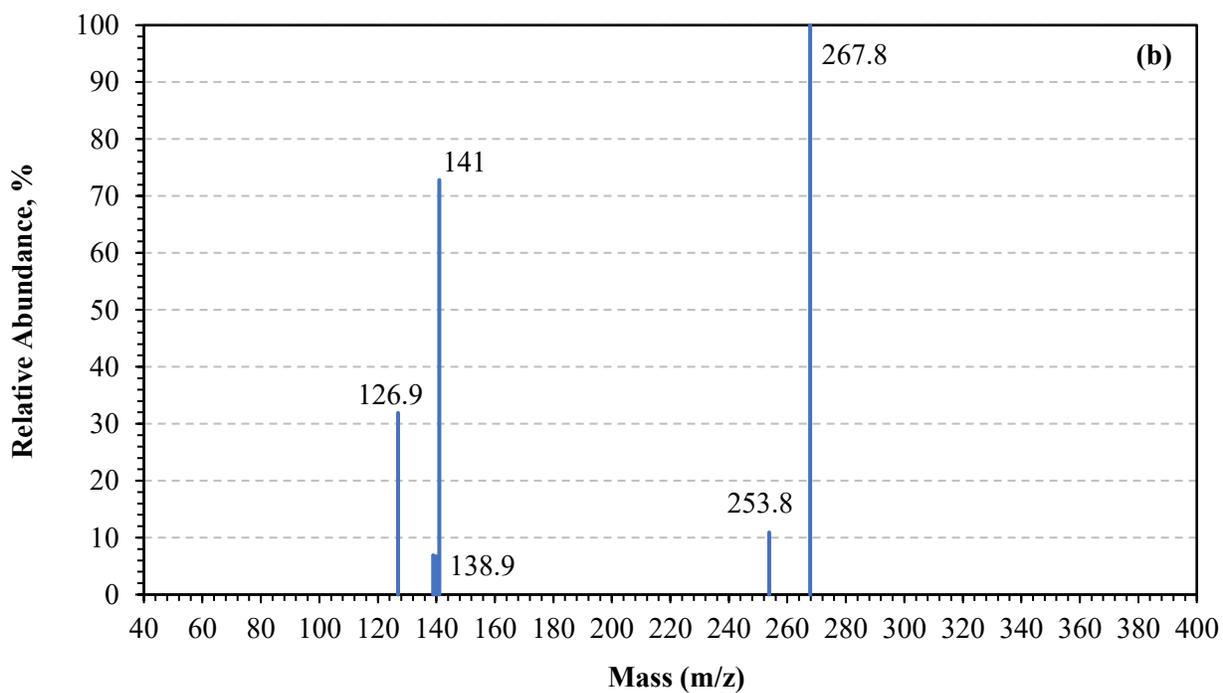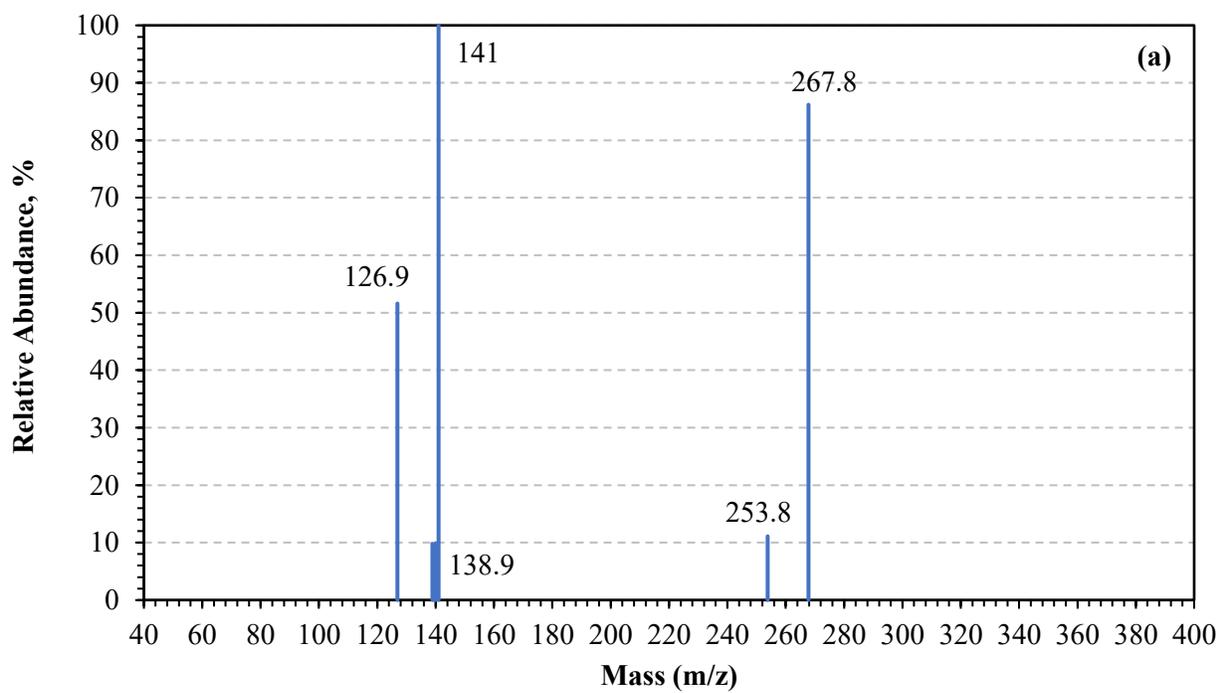Figure D.2. Mass spectra of chloroiodomethane-d$_2$ (internal standard) from analytical standard.

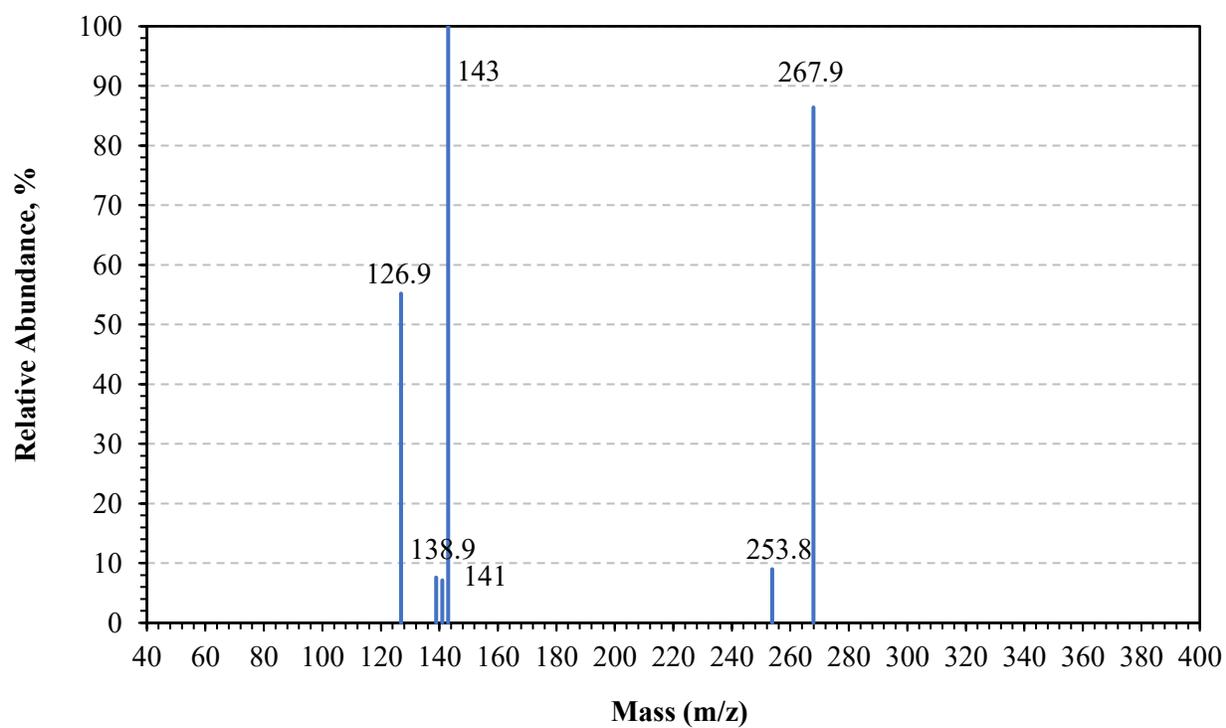Figure D.3. Mass spectra of diiodomethane from (A) analytical standard and (B) BAF samples.

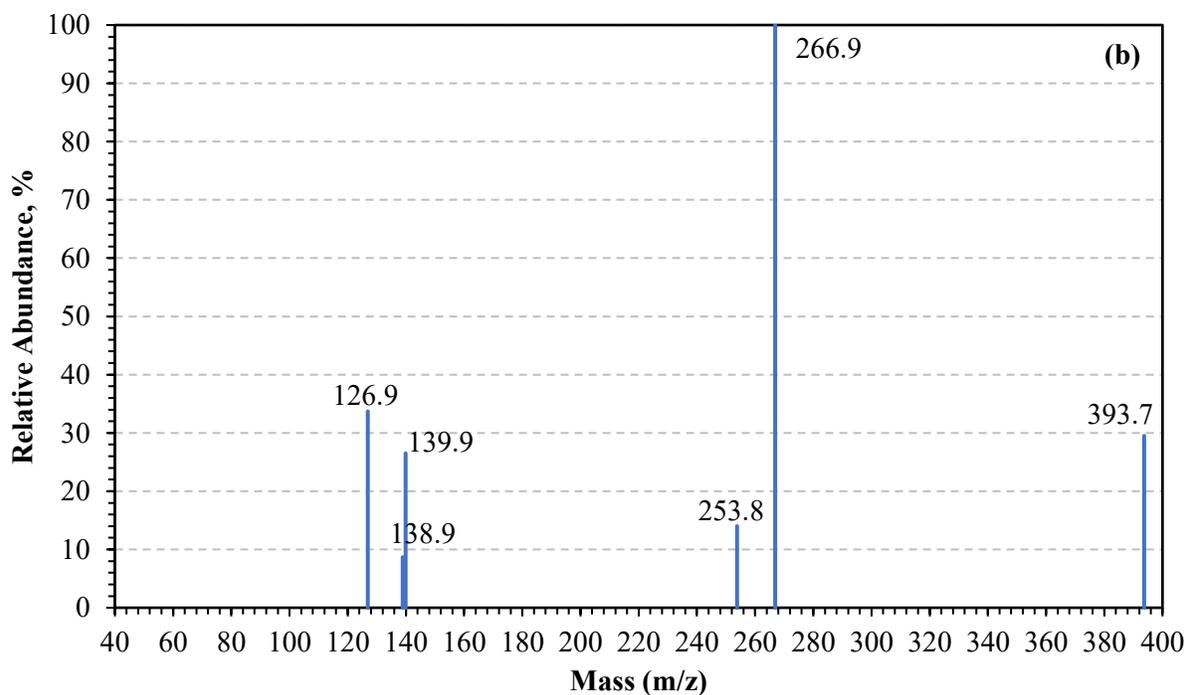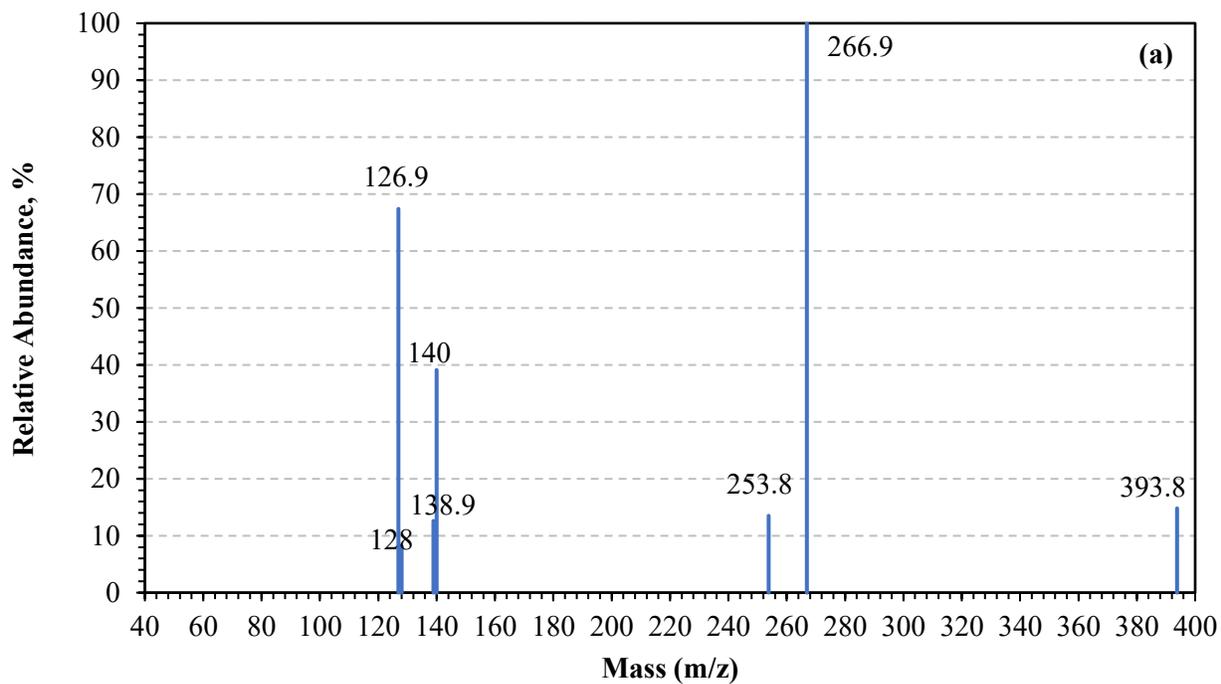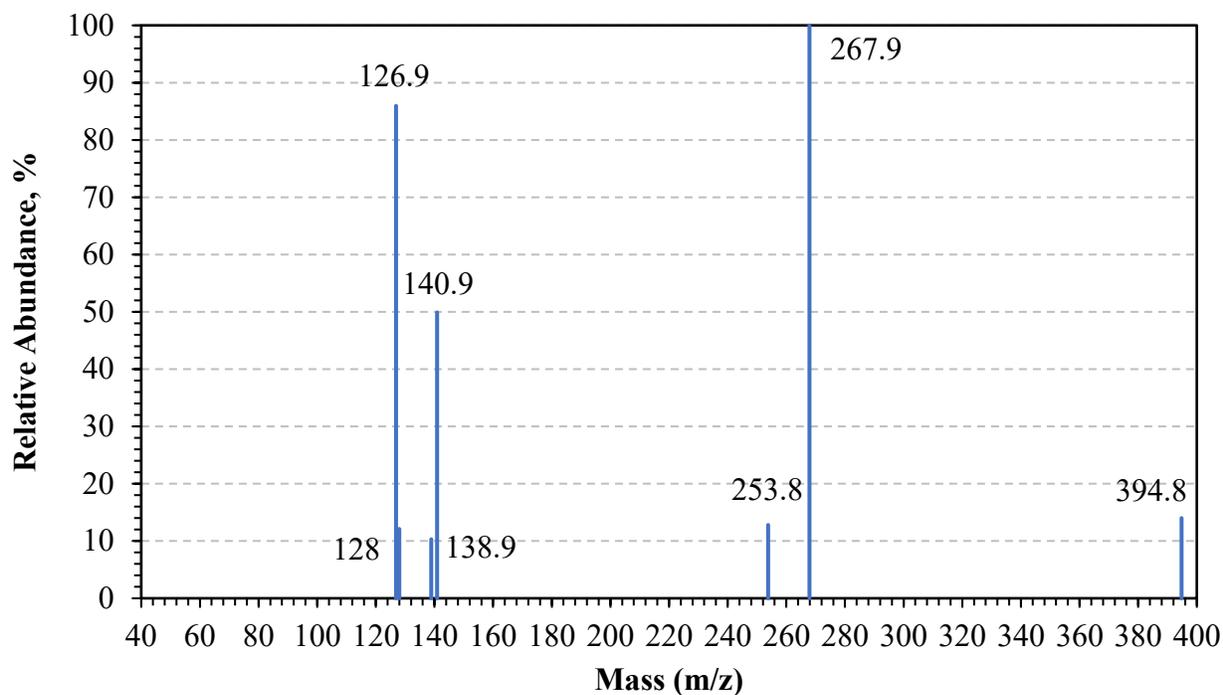Figure D.4. Mass spectra of diiodomethane-d$_2$ (internal standard) from analytical standard.

Figure D.5. Mass spectra of triiodomethane from (A) analytical standard and (B) BAF sample.

Figure D.6. Mass spectra of triiodomethane-d from analytical standard.
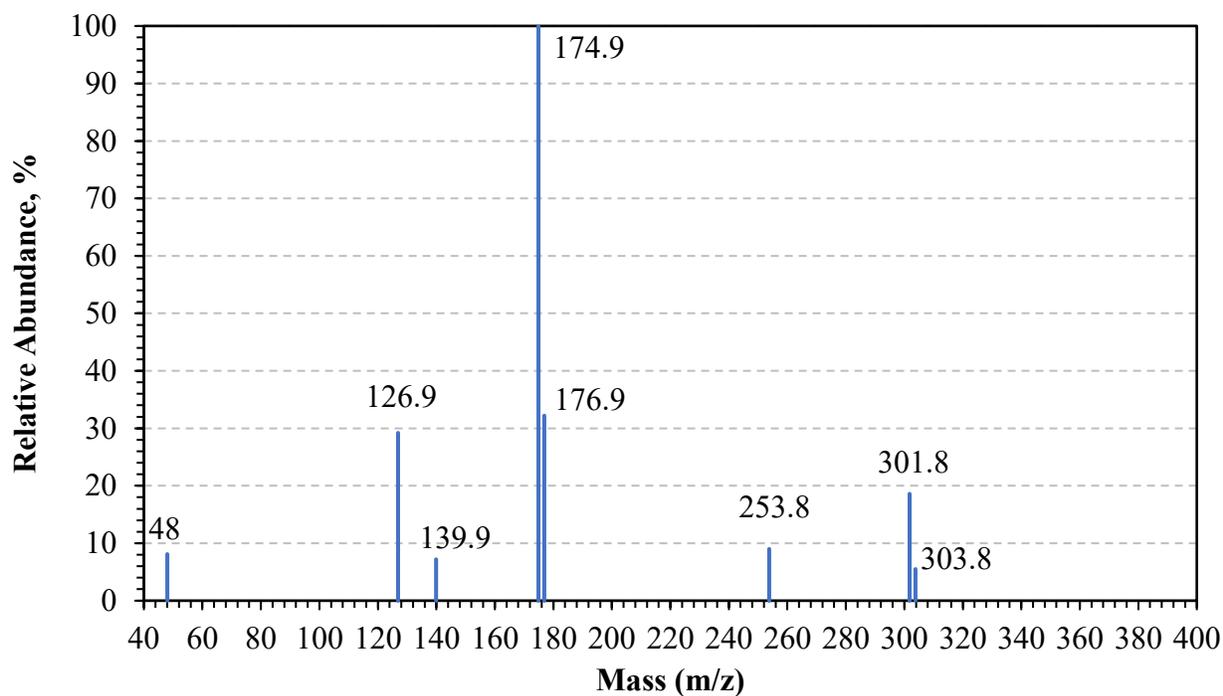

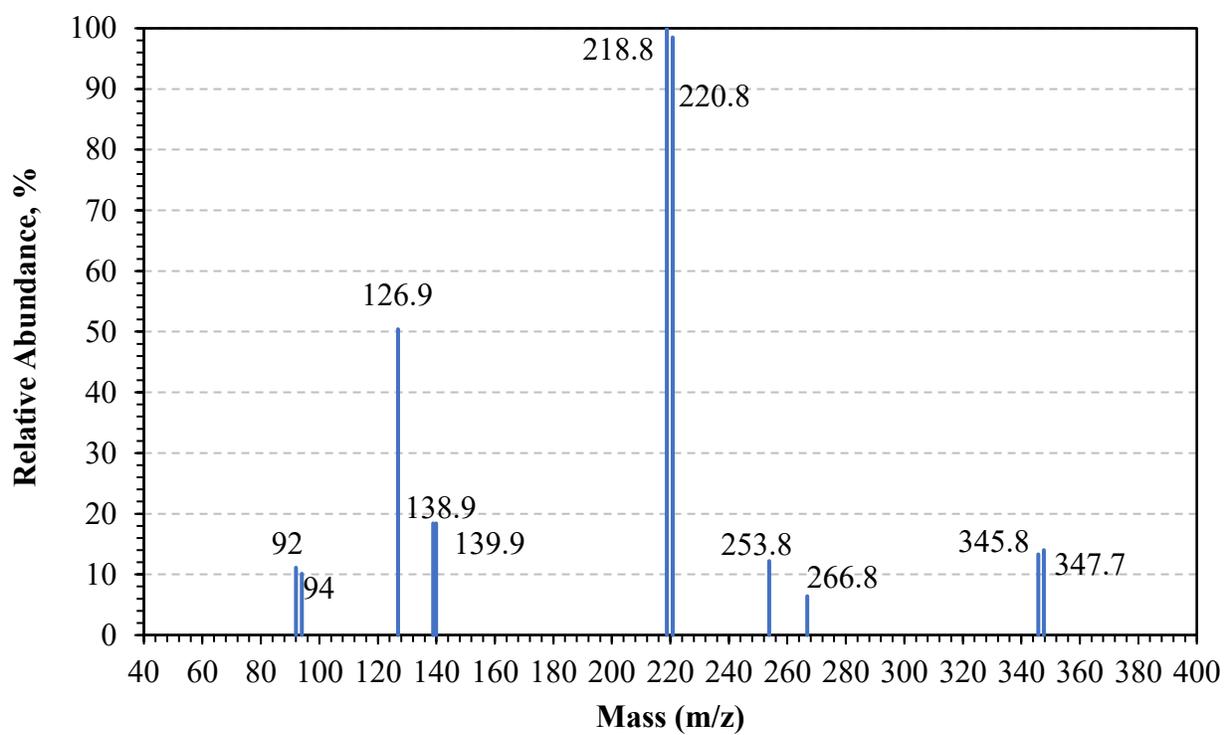Figure D.7. Mass spectra of chlorodiiodomethane from BAF samples.

Figure D.8. Mass spectra of bromodiiodomethane from BAF samples.