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Abstract
The aim of this work was to study the formation of haloacetamides (HAcAms) and other DBPs during chlorination and chloramination of algal organic matter (AlOM). The HAcAms formation potentials of different precursors (amino acids, simulated algal blooms grown in the Red Sea) were evaluated. Experiments with simulated algal blooms were conducted in the presence of bromide ion (synthetic seawater containing 800 µg/L Br^-) to assess the formation of brominated analogues of HAcAms in conditions close to the disinfection of real seawater. Chlorination produced more HAcAms than chloramination from real algae (Synecococcus sp.), thus indicating that the nitrogen of HAcAms comes predominantly from DON through the decarboxylation of amino acids rather than from NH_2Cl. Dibrominated species of DBPs (i.e., DBAcAm, DBAA, DBAN) were the dominant species formed by both chlorination and chloramination of algal blooms samples. Chloramination of the amino acid asparagine produced an important amount of DCAcAm as compared to chlorination, indicating the existence of a specific reaction pathway.

Keywords
Chlorine; chloramines; disinfection byproducts; algal organic matter; haloacetamides

INTRODUCTION

Prechlorination is used in seawater desalination processes to control biofouling in both thermal plants and membrane plants. Important amounts of disinfectant can be required to maintain a residual during algal blooms events. Many disinfection byproducts (DBPs) are formed during seawater pretreatment processes using chlorination, including the regulated trihalomethanes (THMs) and haloacetic acids (HAAs). Nitrogenous DBPs (N-DBPs) generally form in much smaller amounts than regulated DBPs, but have been a growing concern because of their greater health risk (Plewa et al., 2004; Muellner et al., 2007). *In vitro* mamallian cell tests demonstrated that haloacetonitriles (HANs), halonitromethanes (HNMs) and haloacetamides (HAcAms) are more cytotoxic and genotoxic (up to 2 orders of magnitude) than non-nitrogenous THMs and HAAs (Plewa et al., 2008).

As an alternative disinfectant, monochloramine (NH_2Cl) can be used in cooling systems to get a stable disinfectant residual. However, the use of NH_2Cl can be a source of nitrogen and leads to the formation of N-DBPs. Moreover, the disinfection of seawater can produce important amounts of N-DBPs, especially during algal blooms, enriched in nitrogen-containing compounds (e.g., amino acids). Arid regions produce water by desalination of seawater or brackish water, characterized by elevated bromide and iodide ions contents. The presence of high concentrations of bromide (60 mg Br^-/L in the Red Sea) and iodide (0.05 mg I^-/L) ion in seawater favors the formation of brominated and iodinated byproducts that are often more toxic than their chlorinated analogues (Richardson et al., 2007, Richardson et al., 2008).

Haloacetamides are N-DBPs that exert high levels of toxicity compared to other DBPs, but their formation mechanisms are unclear. They are known to be products of the hydrolysis of HANs (Reckhow et al., 2001), however they were reported to be formed independently by chlorination and chloramination of natural organic matter (NOM) (Huang et al., 2012).

Two reaction pathways have been proposed for the formation of haloacetamides during water disinfection. The first one is the decarboxylation pathway, producing haloacetonitriles that are then hydrolyzed into haloacetamides. In this case, the nitrogen atom of haloacetamides is coming from the precursor compound (i.e., amino acids, organic nitrogen) (Huang et al., 2012). The second reaction pathway is the aldehyde pathway, where NH_2Cl reacts with aldehydes to produce HANs and HAcAms (Kimura et al., 2013). In this case, the nitrogen of HAcAms is incorporated from NH_2Cl. Literature about the formation mechanism of HAcAms and their precursors in NOM, wastewater, algal organic matter (AlOM) or seawater organic matter is limited. The HAcAm formation potential of some amino acids (Chu et al., 2010, Huang et al., 2012), humic acids, wastewater effluent and algal EPS (Huang et al., 2012) and culture bacteria (Huang et al., 2013) were reported but no studies were focused on seawater algal organic matter.

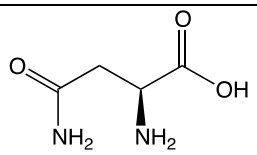
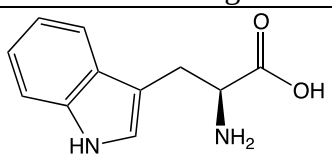
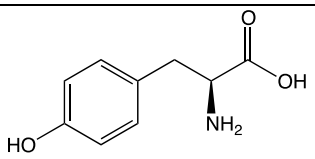
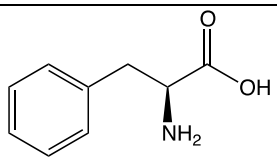
This study investigates the formation mechanisms of haloacetamides during chlorination and chloramination of algal organic matter (AlOM). The aim was to evaluate the importance of the decarboxylation and the aldehyde pathway from different precursors (amino acids, simulated algal blooms). Experiments with simulated algal blooms were conducted in the presence of bromide (synthetic seawater containing 60-65 mg/L Br^-) to assess the formation of brominated analogues of haloacetamides.

MATERIALS AND METHODS

Materials

All experiments were conducted using deionized water (Milli-Q, Millipore) buffered with a mixture of sodium phosphate monobasic and sodium phosphate dibasic. Solution pH values were adjusted as needed using sodium hydroxide or sulfuric acid (0.1 N, Fisher Scientific). Fisher Scientific MTBE and ethyl acetate (>99%) were used without further purification. Amino acids (L-asparagine >98%, L-tyrosine >99%, L-tryptophan >99% and phenylalanine >99%) were used without further purification and were supplied through Sigma-Aldrich (Figure 1). Sodium hypochlorite (NaOCl, 5.65-6%, Fisher Scientific) and ammonium chloride (Acros Organics, 99.6%) were used as chlorination reagents. Ammonium-^{15}N-chloride was purchased from Sigma-Aldrich. Sodium thiosulfate (Fisher Scientific) was used to quench residual oxidant. A mixed standard containing haloacetonitriles (HANs), trichloronitromethane (TCNM) and haloketones (HKs) (EPA 551B Halogenated Volatiles Mix), a mixed standard containing 9 HAAs (EPA 552.2 Methyl Ester Calibration Mix) and surrogate standard decafluorobiphenyl (99%) were supplied from Supelco (Sigma-Aldrich). Chloro-, bromo- dichloro- and trichloroacetamide were obtained from Sigma-Aldrich. Other haloacetamides (HAcAms) were purchased from Cansyn Chem. Corp.

Table 1. Molecular structures of investigated amino acids

			
L-asparagine	L-tryptophan	L-tyrosine	Phenylalanine

Preparation and analysis of chlorine and chloramine

Monochloramine (NH_2Cl) stock solutions (50 mM) were prepared fresh daily by dissolving ammonium chloride (NH_4Cl) in deionized water adjusted to pH = 8.5 with sodium hydroxide. Sodium hypochlorite (NaOCl) was then added slowly to the rapidly stirred solution, at a Cl:N molar ratio of at least 1:1.2 to avoid breakpoint chlorination resulting from local excess of hypochlorite. Adjusting the pH at 8.5 minimizes the disproportionation of NH_2Cl to dichloramine ($NHCl_2$), since $NHCl_2$ forms at pH < 8 according to equilibrium 1:

$$2NH_2Cl + H^+ = NHCl_2 + NH_4^+ \quad (1)$$

NH_2Cl and $NHCl_2$ were quantified in stock solutions by monitoring absorbances at their respective λ_{max} (λ_{NH2Cl} = 245 nm; λ_{NHCL2} = 295 nm). Residual oxidant in samples after experiments was analysed iodometrically (Standards Methods for the Examination of Water and Wastewater, 1995).

Experimental methods

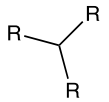
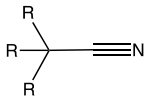
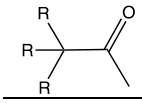
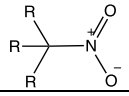
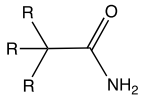
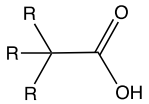
All glassware used during these experiments was washed and baked at 500 °C for at least 5 h prior to use. Reactions were conducted in sealed 65 mL amber glass bottles at room temperature (20 °C). Chloramination experiments were conducted following the approach of Krasner et al. (2004), using an excess of oxidant (15 mg/L as Cl_2) and a reaction time of 72 hours at pH 8.

Simulated algal blooms grown directly in the Red Sea (mesocosms) were employed as algal source (main species Synecococcus sp.). Aqueous solutions were prepared by dilution of seawater samples (simulated algal blooms) with synthetic seawater (Grassoff et al., 1976) to the desired DOC concentration (1.5 mg C/L). Amino acids solutions were prepared by dissolving a pre-determined amount of amino acids in Milli-Q water containing 10 mM carbonate buffer. 275 µL of preformed monochloramine was then added. Each series of experiments included a blank (buffered Milli-Q water) and each sample was triplicated. After 72h of contact time, 5 mL of samples were used for residual chlorine analysis and 3 x 20 mL were used for DBPs analysis. Molar yields were calculated based on the molar concentrations of amino acids (mol DBP formed × 100 / mol initial compound).

DBP analysis

4 THMs, 4 haloacetonitriles (HANs), 2 haloketones (HKs) and chloropicrin were extracted following EPA method 551, which consists in a liquid-liquid extraction using MTBE, followed by detection using an Agilent 7890A gas chromatograph coupled with electron capture detector (GC-ECD) (Munch et al., 1995a). 9 HAAs were extracted and analyzed following EPA method 552.2, which is based on a liquid-liquid extraction with MTBE in acidic conditions followed by derivatization to methyl esters using acidic methanol (Munch et al., 1995b). Extracts were analysed using an Agilent 7890A GC system coupled with a 5975C mass spectrometer (GC-MS). HAcAms were analysed following the same protocol than EPA method 551, but using ethyl acetate for the liquid-liquid extraction step. All DBPs analysed are described in Table 2.

Table 2. Description of monitored DBPs

Method of determination	Class of compounds and structures	Abbreviation	Full name
EPA 551	Trihalomethanes (THMs) 	TCM	Trichloromethane (chloroform)
		DCBM	Dichlorobromomethane
		DBCM	Dibromochloromethane
		TBM	Tribromomethane (bromoform)
	Haloacetonitriles (HANs) 	DCAN	Dichloroacetonitrile
		TCAN	Trichloroacetonitrile
		BCAN	Bromochloroacetonitrile
		DBAN	Dibromoacetonitrile
	Haloketones (HKs) 	1,1-DCP	1,1-dichloropropanone
		1,1,1-TCP	1,1,1-trichloropropanone
	Halonitromethane 	TCNM	Trichloronitromethane
EPA 551 - EA	Haloacetamides (HAcAms) 	CAcAm	Chloroacetamide
		BAcAm	Bromoacetamide
		DCAcAm	Dichloroacetamide
		TCAcAm	Trichloroacetamide
		DBAcAm	Dibromoacetamide
		TBAcAm	Tribromoacetamide
		BCAcAm	Bromochloroacetamide
		CIAcAm	Chloroiodoacetamide
		BIAcAm	Bromoiodoacetamide
		DIAcAm	Diiodoacetamide
EPA 552.2	Haloacetic acids (HAAs) 	MCAA	Monochloroacetic acid
		MBAA	Monobromoacetic acid
		DCAA	Dichloroacetic acid
		TCAA	Trichloroacetic acid
		BCAA	Bromochloroacetic acid
		DCBAA	Dichlorobromoacetic acid
		DBAA	Dibromoacetic acid
		DBCAA	Dibromochloroacetic acid
		TBAA	Tribromoacetic acid

RESULTS AND DISCUSSION

Formation of haloacetamides from amino acids

The formation of haloacetamides was investigated after 72h of chlorination and chloramination (15 mg/L as Cl_2) of 4 amino acids (50 µM each) at pH 8 (Figure 1). Asparagine was found to be a major dichloracetamide (DCAcAm) precursor during chloramination, producing up to 1160 µg/L DCAcAm. This can be attributed to the amide group present in the asparagine structure (Table 1). However, this does not explain why chloramination formed much more DCAcAm than chlorination. Other amino acids were much lower precursors of DCAcAm. In the case of tyrosine and tryptophan, more DCAcAm was formed by chlorination. These results indicate that different reaction pathways

occur during chlorination and chloramination of amino acids. Especially, a specific pathway seems to occur during the chloramination of asparagine.

Huang et al. (2012) proposed a formation mechanism to explain the formation of DCAcAm from asparagine, but did not describe a specific pathway for NH_2Cl, because the formation of DCAcAm was similar by chlorination and by chloramination in their experimental conditions (100 µM asparagine, 10 mg/L as Cl_2, t = 2h, pH 6.9).

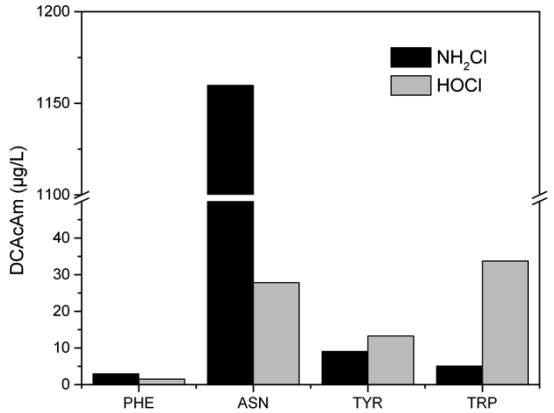


Figure 1. DCAcAm formation potential from chlorination and chloramination (15 mg Cl_2/L) of amino acids (50 µM) after 72h contact time at pH8 (10 mM carbonate buffer).

TCAcAm formation was more important than DCAcAm formation during chlorination of amino acids, indicating a higher incorporation of chlorine atoms because of a higher chlorine transfer from HOCl to haloacetamides (Figure 2.a). Tryptophan was the most important precursor of TCAcAm from chlorination, with up to 115 µg/L of TCAcAm formed. During chloramination, levels of TCAcAm formed were very low and similar for each amino acid (i.e., 4.5 – 5 µg/L), indicating lower chlorine incorporation during chloramination.

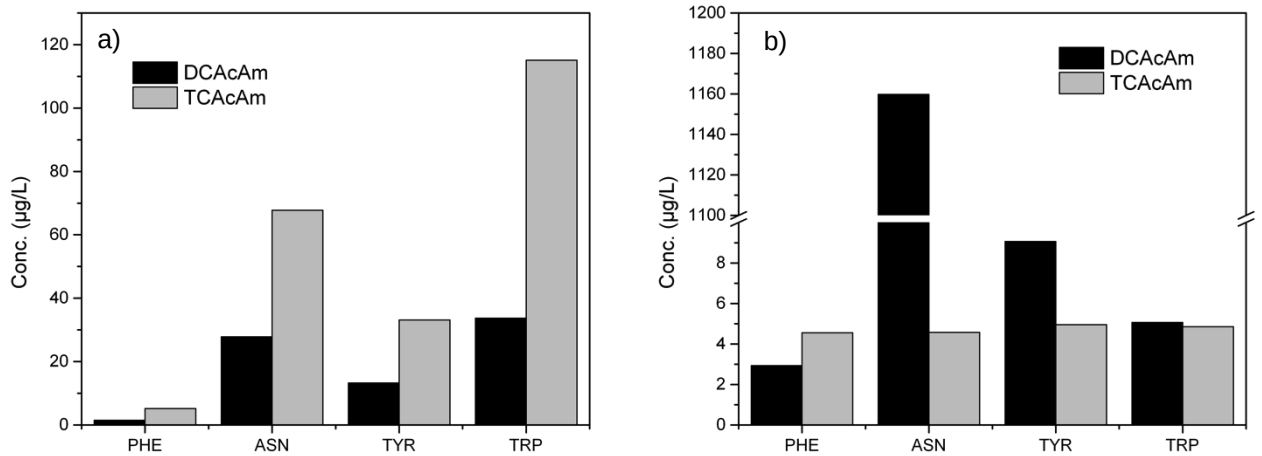


Figure 2. Formation of DCAcAm and TCAcAm from a) chlorination and b) chloramination of amino acids ([amino acids] = 50 µM; [oxidant] = 15 mg/L as Cl_2; t = 72h; pH = 8).

Formation of haloacetamides from simulated algal blooms

Simulated algal blooms grown directly in the Red Sea (8000 L mesocosms) were employed as algal source (main species Synecococcus sp.) for DBPs formation potential tests. Four different mesocosms

were studied, each consisting in a different set of nutrients conditions (1: nitrates and phosphates added every day; 2: nitrates and phosphates added once; 3: nitrates, phosphates and silica added every day; 4: nitrates, phosphates and silica added once). All four samples were diluted with synthetic seawater to equal concentrations of DOC (1.5 mg C/L) and were chlorinated and chloraminated (5 mg Cl_2/L) in the lab during 72h. Results showed a higher formation of haloacetamides by chlorination than by chloramination (Figure 3.a). The first mesocosm formed the highest concentration of HAcAms (240 µg/L), probably because of the presence of a higher concentration in dissolved organic nitrogen (DON = 0.19 mg N/L in samples from mesocosm 1 and 3; DON = 0.12 mg N/L in samples from mesocosms 2 and 4) due to continuous addition of nitrates. These results indicate that the nitrogen atom of haloacetamides would predominantly originate from organic nitrogen contained in algal cells, and not from NH_2Cl. This implies that the predominant mechanism of HAcAms formation from algae cells is the decarboxylation pathway, where the nitrogen atom of HAcAms originates from DON. This is also suggested by the similar trend in HANs formation, exhibiting higher concentrations from chlorination than from chloramination (Figure 3.b). In the decarboxylation pathway, HAcAms are produced through HANs hydrolysis. Hence our results suggest a similar pathway for HANs and HAcAms formation, favoured by the presence of free chlorine.

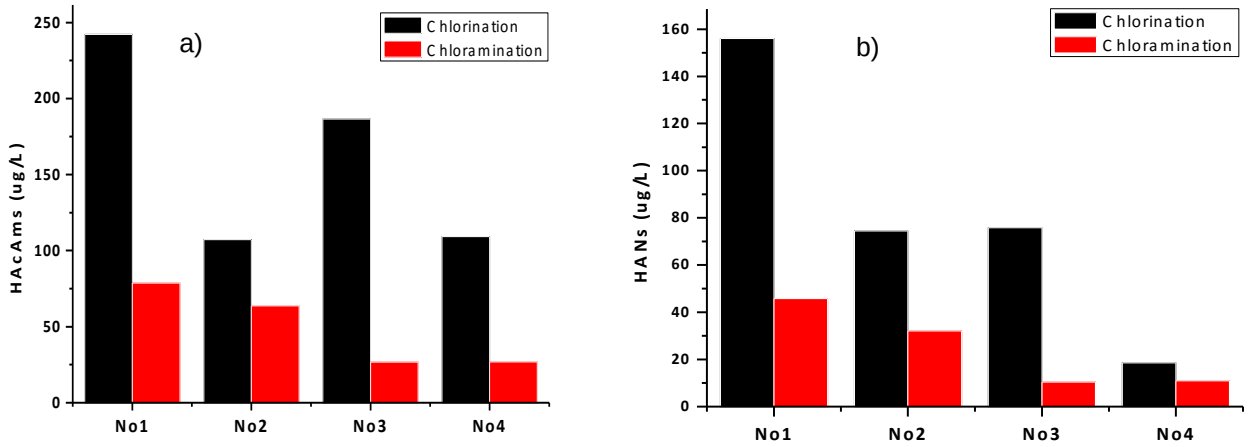


Figure 3. Formation of a) total HAcAms and b) total HANs from chloramination and chlorination (5 mg/L as Cl_2) of four simulated algal blooms (mesocosms) (1.5 mg C/L) during 72h.

As it could be expected because of the presence of bromide ion in the seawater samples (60-65 mg/L as Br^-), the proportion of brominated DBPs formed after chlorination and chloramination was more important than the amount of chlorinated DBPs. TBM, DBAcAm, DBAA, and DBAN were the predominant species among THMs, HAcAms, HAAs and HANs, respectively. This is explained by the fast reaction between chlorine and bromide ion leading to the production of hypobromous acid (HOBr) (Equation 2), which is an oxidizing species even more reactive than hypochlorous acid (HOCl) (Heller-Grossman et al., 1993).

$$HOCl + Br^- \rightarrow HOBr + Cl^- \quad (2)$$

CONCLUSIONS

The aim of our work was to study the formation of HAcAms from chlorination and chloramination of marine algal organic matter. DBP formation potential tests were performed from model compounds (amino acids) and from simulated algal blooms in real seawater conditions. Results show that chlorination produces more HAcAms than chloramination from real algae (Synecococcus sp.), thus indicating that the nitrogen of HAcAms comes predominantly from DON through the decarboxylation of amino acids rather than from NH_2Cl. Results of HAcAm formation potentials

from amino acids are in agreement with this pathway, tyrosine and tryptophan exhibiting higher DCAcAm formation by chlorination than by chloramination. However, asparagine was found to be a major DCAcAm precursor during chloramination, thus indicating a specific reaction pathway. While the amide group of asparagine is probably the main reason of its important DCAcAm formation potential, the specific role of NH_2Cl as compared to HOCl needs to be further investigated. The use of isotopically labelled monochloramine($^{15}NH_2Cl$) could help understanding the incorporation of nitrogen from asparagine or from NH_2Cl.

Dihalogenated species of DBPs (i.e., DBAcAm, DBAA, DBAN) were the dominant species formed by both chlorination and chloramination of mesocosms samples, while chlorination of amino acids formed more TCAcAm than DCAcAm, indicating a higher incorporation of chlorine.

Future work will focus on the formation of haloacetamides by chloramination of different marine AlOM obtained from algae monocultures grown in laboratory to understand the specificities of haloacetamides precursors from real algae. Reactions will be conducted in the presence isotopically labelled $^{15}NH_2Cl$ to assess the proportion of nitrogen coming from organic nitrogen sources and from monochloramine.

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