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Additional Information

Impact of chlorination and pre-ozonation on disinfection by-products formation from aqueous suspensions of cyanobacteria: Microcystis aeruginosa, Anabaena aequalis and Oscillatoria tenuis

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Abstract

The influence of the pre-ozonization on the formation of disinfection by-products (DBPs) upon chlorination for fresh waters containing three common cyanobacteria, namely Microcystis aeruginosa, Anabaena aequalis and Oscillatoria tenuis at 10,000 cells/mL is reported. Specifically, the formation carbonaceous-DBPs (C-DBPs) (trihalomethanes (THMs), haloacetic acids (HAAs) and haloketones (HKs)) and nitrogenous-DBPs (N-DBP) (haloacetonitriles (HAN) and trichloronitromethane (TCNM)) has been determined as a function of the pH (6.5 or 8.0 and bromide ion concentration (300 μg/L). The main C-DBPs were THMs and HAAs with negligible formation of HKs accompanied by minor amounts of HANs in the absence of TCNM. Pre-ozonation of the aqueous cyanobacteria suspensions does not allow a control over all the DBPs. In fact, pre-ozonation increases THM formation and generates TCNM, has low influence on HAAs and only decreases the formation of HANs. The overall conclusion of this work is that pre-ozonation of waters containing a relatively low concentration of common fresh water cyanobacteria is not an appropriate process to decrease DBP formation from chlorine. Cyanobacteria removal from raw water before chlorination or ozonation should reduce DBP formation.

Keywords: cyanobacteria; chlorination; ozonation: disinfection by-products

1. Introduction

Cyanobacteria, traditionally identified as blue-green algae, occur worldwide in fresh and marine natural aquatic ecosystems exposed to sunlight, allowing the operation of oxygenic photosynthesis necessary for the metabolic synthesis of carbohydrates from CO_2(Merel et al. 2013). One of the main concerns related with the presence of cyanobacteria in water is the production of toxins for aquatic or terrestrial organisms, animals and humans with unwanted effects by contact or ingestion, including hepatoxocity and tumor promotion (Carmichael and Boyer 2016, Codd et al. , Falconer 1991, Frazier et al. 1998, Hawkins et al. 1985, Jochimsen et al. 1998, Turner et al. 1990). In the conventional drinking water treatment, the presence of cyanobacteria bloom impedes the settling process and increases the demand of oxidant employed as water disinfectant during the treatment (Chen et al. 2017).

In addition, previous studies have reported that cyanobacteria aqueous suspensions are important precursors of disinfection by-products (DBPs) during water chlorination (Goslan et al. 2017). In this context, Microcystis aeruginosa has been the preferred cyanobacteria for these studies due to its ubiquity in the surface waters (Daly et al. 2007, Fang et al. 2010, Ho et al. 2006, Zhu et al. 2015), among others. Most of these studies, however, have focused on the total formation of few DBPs, but they do not provide the individual evaluation of each of the various C-DBPs and N-DBPs. For example, some studies have studied the impact of cyanobacteria aqueous suspensions chlorination on the formation of individual families of DBPs such as trihalomethanes (THMs) (Shi et al. 2019), haloacetic acids (HAAs)(Ge et al. 2011) or the concomitant production of few DBPs such as haloacetonitriles (HANs)/THMs(Pu et al. 2013) or THMs/HAAs(Hong et al. 2008) among others. It is noteworthy that the formation of unregulated nitrogenous-DBPs (N-DBPs), such as HANs or trichloronitromethane

(TCNM) is highly important since some of these DBPs are more genotoxic, cytotoxic or carcinogenic than carbonaceous-DBP (C-DBPs) and the presence of phytoplankton in the raw water increases their formation (Shah and Mitch 2012) such as THMs or HAAs(Plewa et al. 2004).

Another frequent situation found in the studies of DBP formation from cyanobacteria aqueous suspensions is that some reports have focused on the exclusive formation of chlorinated-DBPs at pH 7. Thus, there is still room to investigate the impact of the presence of bromide ions(Ge et al. 2011, Hong et al. 2008, Plummer and Edzwald 2001, Shi et al. 2019) at the concentration range naturally occurring and the influence of the pH(Shi et al. 2019) during the chlorination of the water contaminated by cyanobacteria. Nowadays it is well-stablished that the impact of some brominated DBPs such as THMs, HAAs, HANs on the human health is higher compared to their corresponding chlorinated analogues (Yang et al. 2014). Brominated DBPs are more cytotoxic and genotoxic than their corresponding chlorinated analogues (Bond et al. 2011). In addition, regardless the higher occurrence and concentration of THMs and HAAs in drinking waters, N-DBPs such as HANs seem to be the responsible DBPs for toxicity (Plewa et al. 2017). Regarding the influence of the pH, most of studies related with cyanobacteria chlorination have been conducted at about pH 7(Hong et al. 2008, Plummer and Edzwald 2001, Shi et al. 2019) while most of the surface natural waters have pH values around 8. At pH 8 the main chlorine form is ClO^-, while at pH 7 the main form is HClO (Deborde and von Gunten 2008). Chlorine speciation influences in large extent DBP formation. Therefore, it is desirable to obtain further information on the influence of the presence of bromide ions and pH on DPB formation during the chlorination of some common cyanobacteria found in fresh waters destined for human consumption.

One of the main strategies employed to minimize the risk of cyanobacteria accumulation in drinking water treatment plants and degrade the toxins released upon cell lysis is the use of ozone as oxidant (Daly et al. 2007, Fan et al. 2014). In addition, ozone has been generally employed as pre-oxidant to minimize DPB formation in the subsequent water chlorination (Richardson et al. 2007). In this context, the number of studies reporting the influence of pre-ozonation of cyanobacteria aqueous suspensions on DPB formation in the chlorination is much lower respect to those limiting only to chlorination (Plummer and Edzwald 2001). Thus, it would be desirable further gain information on the influence of pre-ozonation also for other common cyanobacteria in water on the formation of DBPs, particularly in the presence of bromide ions that can form brominated THMs performing the experiments at pH values commonly found in natural waters.

Towards this goal, the present study shows the impact of the presence of three common cyanobacteria in surface waters, namely, Microcystis aeruginosa, Anabaena aequalis and Oscilatoria tenuis on DBP formation upon chlorination with or without a pre-ozonation process at relatively low cell concentration (10,000 cells/mL). In particular, THM, HAA, HAN, haloketone (HK) and chloropicrin or trichloronitromethane (TCNM) formation together with the chlorine demand has been evaluated in the absence and presence of bromide ions as a function of the pH.

Considering the ways in which the presence of cyanobacteria can influence DBP, it has been reported that each class of biomolecule has a different behaviour regarding DBP formation. Thus, some of us have reported that chlorination of carbohydrate aqueous solutions result in a moderate chlorine demand (~1.5 mg Cl_2/mg_C at pH 8 at 20 ºC after 3 d) with most of consumed chlorine atoms ending as THMs (100 μg/L) (Navalon et al. 2008).

Other studies have reported that the presence of N-containing organic compounds such as amino acids or peptides (Hureiki et al. 1994) results in some cases in chlorine demands higher than humic substances (Hong et al. 2009). For example, some some peptides and proteins exhibit chlorine demand between 3-4 mg Cl_2/mg_C (Hureiki et al. 1994). In general, chlorination of N-containing organic compounds results in the formation of C-DBPs including THMs and HAAs, together with lesser amounts of aldehydes and HKs. In this context, it is pertinent to mention that in the case of the cyanobacteria Oscillatoria tenuis studied in this work, the main amino acids present in its composition are in average aspartic acid (7.33 %), glutamic acid (10.1 %), leucine (7.34 %), histidine (1.65 %) and methionine (0.82 %).(Hong et al. 2008) Of note is that aspartic acid have been identified as one of the main non-aromatic HAA precursors (83.2 μg mg^{-1} C) present in the organic matter of natural waters. In addition, N-DBPs(Bond et al. 2011) such as organic haloamines(Hureiki et al. 1994), halonitriles, haloamides, halonitro compounds or cyanogen chloride are also formed upon chlorination of N-containing organic compounds (Trehy et al. 1986). As commented in the introduction, HANs and TCNM are important N-DBP groups due to their high geno- and cytotoxicity compared to regulated C-DBPs such as THMs or HAAs(Plewa et al. 2004).

Regarding to DBP formation upon chlorination of lipids it has been reported that the higher number of double bonds in their structure the higher DBP formation (Deborde and von Gunten 2008). For example, chlorination of common algal fatty acids showed that the higher amounts of double bonds the higher amount of TCNM and DCAA(Liang et al. 2012). The reader is referred to some existing reviews and articles for a deeper discussion regarding chlorination mechanisms leading to DBP formation as a function of the structure of the precursor(Deborde and von Gunten 2008).

Overall, the results obtained indicate that chlorination of fresh water contaminated by three common cyanobacteria at relatively low cell concentration results in significant increase in DBP formation. In addition, a pre-ozonation of waters containing these three cyanobacteria is not useful to decrease the concentration of C-DBPs and N-DBPs. Our study indicates the need to remove cyanobacteria from the raw water before ozone pre-oxidation and/or chlorination in order to observe a diminution in the DBP formation.

2. Materials and methods

2.1. Reagents

All the reagents employed in this work were of analytical or HPLC grade. The list of the reagents includes: a) EPA 501/601 THMs calibration mix (2000 µg/mL each component in methanol; Merck) for the analysis of chloroform (TCM), dichlorobromomethane (BDCM), dibromochloromethane (DBCM) and bromoform (TBM); b) HAA standard mixture (EPA 552.2 Methyl Ester Calibration Mix, Sigma-Aldrich) for the analysis of monochloro-, dichloro-, trichloro-, monobromo-, dibromo-, bromochloro-, bromodichloro-, dibromochloro-, and tribromo-acetic acids (MCAA, DCAA, TCAA, MBAA, DBAA, BCAA, BDCAA, DBCAA, and TBAA, respectively); c) EPA 551B halogenated volatiles mix (2000 µg/mL each component in acetone; Sigma-Aldrich) for the analysis of bromochloroacetonitrile (BCAN), dibromoacetonitrile (DBAN), dichloroacetonitrile (DCAN), 1,1-dichloro-2-propanone (1,1-DCP), 1,1,1-trichloroacetone (1,1,1-TCP), trichloroacetonitrile (TCAN), trichloronitromethane (TCNM). d) Sodium hypochlorite (NaOCl) (5 % active chlorine, Acros Organics). The three cyanobacteria under study Microcystis aeruginosa, Anabaena aequalis and Oscillatoria tenuis were supplied Spanish Bank of Algae (BEA-Banco Español de Algas).

2.2. Experimental procedures and analysis

Supplementary information describes the detailed description of cyanobacteria aqueous suspensions preparation (Section S1), the pre-ozonation and chlorination experiments (Section S2) and the analytical methods (Section S3) employed in this study. Herein, in contrast to precedents using high Algae concentration of 1,000,000 cells/mL to study DBP formation (Fang et al. 2010), the present work aims to determine the influence of a moderate cyanobacteria concentration on DBP formation upon chlorination, depending on whether or not a pre-ozonation step is performed (Almuhtaram et al. 2018). The range of concentration selected in the present study is more often occurring in water treatment plants, in which the operation conditions makes less probable acute cyanobacteria proliferation.

Optical microscopy confirmed the presence in the aqueous suspension of the three cyanobacteria under study, namely Microcystis aeruginosa, Anabaena aequalis and Oscillatoria tenuis, with their expected morphology (Figure 1).

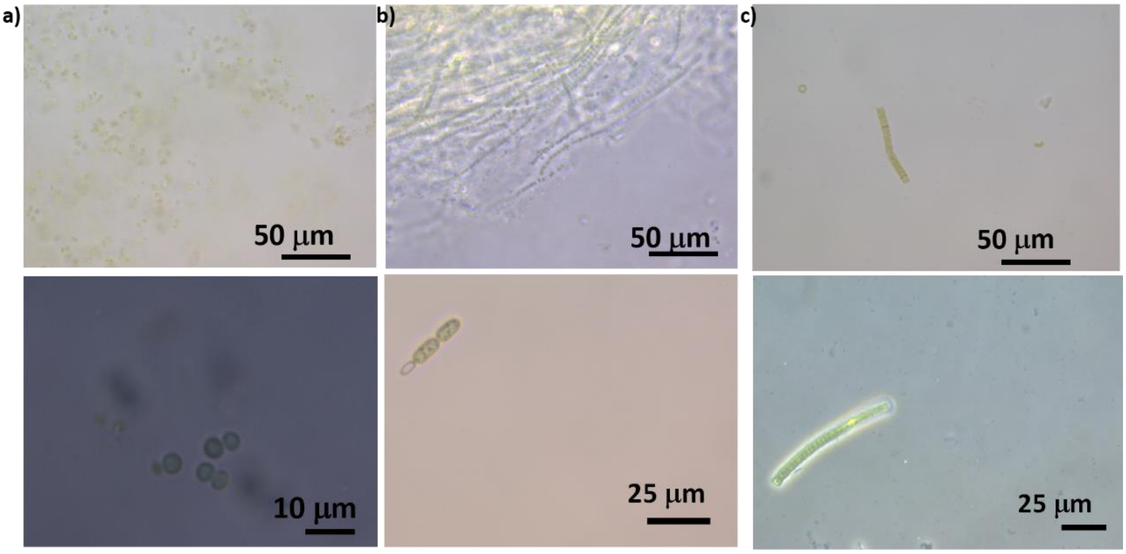

Figure 1. Optical microscopy images of Microcystis aeruginosa (a), Anabaena aequalis (b) and Oscillatoria tenuis (c).

In general, the aqueous cyanobacteria solution (10,000 cells/mL; 200 mL) is ozonated at 1.6 mg/L and contacted for 2 h at pH 8. Then, the pre-ozonated solution is chlorinated (100 mL) under headspace-free conditions with a chlorine dose of 11 mg/L at either pH 8 or 6.5 at 20 ºC for 72 h. More details on the ozonation and chlorination processes can be found in the supplementary information.

2.3. Cyanobacteria characterization

The three cyanobacteria under study were prepared at 10,000 cells/mL as described earlier. Table 1 collects the TOC of the three cyanobacteria suspensions under study as well as their composition in terms of percentage of lipids, proteins and carbohydrates, determined according to the literature(Cao et al. 1997, Sun et al. 1997). It is important to note that previous studies have reported the formation upon chlorination of several DBPs depending on the type of biomolecule either carbohydrates, proteins or lipids as organic model precursors to understand the behaviour of natural organic matter present in fresh water.

Table 1. List of cyanobacteria under study, TOC of the employed suspensions and composition in terms of proteins, carbohydrates and lipids.

	TOC (mg/L)[a]	Carbohydrates (%)	Proteins (%)	Lipids (%)	Ref
Microcystis Aeruginosa[a,b]	1.04	14.5	82.0	3.57	(Cao et al. 1997)
Oscillatoria tenuis[a]	0.7	23.9	64.6	11.6	(Sun et al. 1997)

Anabaena aequalis[a]	1.04	39.8	54.6	5.62	(Cao et al. 1997)

[a] TOC values corresponding to a cell concentration of 10^4 cells/mL

[b] TOC value of 2.4 mg/L at a cell concentration of 10^6 cells/mL

3. Results and discussion

Considering the distribution of biomolecules in the three cyanobacteria under study shown in Table 1 and having in mind that each class of biomolecule renders a different distribution of DBP, it was anticipated that chlorination of the three cyanobacteria aqueous suspensions should follow the expected behaviour respect to C- and N-DBP formation. Thus, in spite of the complexity of the organic components present in the three cyanobacteria under study, the present study attempts to rationalize the observed results on DBP formation upon chlorination based on the course cyanobacteria composition in polysaccharide, proteins and lipid-like organic moieties. Note that according to Table 1, the three cyanobacteria exhibit notable differences in the percentages of the three main types of biomolecules.

3.1. Influence of the presence of cyanobacteria on chlorine demand during chlorination

One of the negative impacts of the presence of algae in the aquatic resources destined to human consumption is the increase of the chlorine demand that has to lead to higher DBP concentration(Zamyadi et al. 2013). Figure 2a shows the chlorine demand of the three cyanobacteria aqueous suspensions (10,000 cells/mL) under study namely Microcystis aeruginosa, Anabaena aequalis and Oscillatoria tenuis at pH values 8 or 6.5

in the absence or presence of bromide ions (300 μg L^{-1}). On one hand, the chlorine demand for the all three cyanobacteria suspensions at pH 8 in the absence of bromide ions is around 6.6 ±0.7 mg L^{-1}. Considering that TOC values of the three cyanobacteria suspensions at 10,000 cells/mL, chlorine demands are 1.04 ±0.2 mg L^{-1} or ~ 6.3 mg free chlorine/mg TOC. The somewhat higher chlorine demand of Microcystis aeruginosa respect to the other two cyanobacteria under study may be partially attributed to its higher protein content (Table 1). These values are in the range of those reported for some N-containing organic compounds, based on amino acids varying from 3.4 to 10 mg/L of C as a function of their chemical structure.

On the other hand, the chlorine demand of the three cyanobacteria under study at pH 6.5 decreases to the average value of 5.3 ±0.5 mg L^{-1} or 5.1 mg L^{-1} chlorine per mg of TOC. These results indicate that the higher the pH value during the chlorination of the cyanobacteria suspensions, the higher chlorine demand. The main chlorine species present in water at pH 8 is ClO^- (E_{RED}^0 = 0.81 eV) that is a worst oxidizing and chlorinating agent than HClO (E_{RED}^0 = 1.49 eV) that is the species present at pH 6.5. Thus, it can be concluded that the experimental higher chlorine demand at basic pH values during cyanobacteria aqueous suspension chlorination should be due to the different organic substances speciation. In this context, it has been reported that saccharide chlorination at basic pH values requires higher chlorine consumption due to the occurrence of oxidation and chlorination compared to the chlorination at acidic pH values (Navalon et al. 2008). Other works have also observed that the chlorine consumption of proteins, peptides and amino acids is also higher at basic pH values due to the N-chlorination pathway that is hampered at acidic pH values in which the amino groups are protonated (Deborde and von Gunten 2008). The chlorination rates of lipids are mainly controlled by the chlorine speciation at the corresponding pH value of the water (Deborde and von Gunten 2008). Overall, it is likely to propose that the higher chlorine demand of cyanobacteria at basic pH values is due to both the reaction of chlorine with deprotonated amino groups and the chlorine reaction with carbohydrates through oxidation and chlorination pathways.

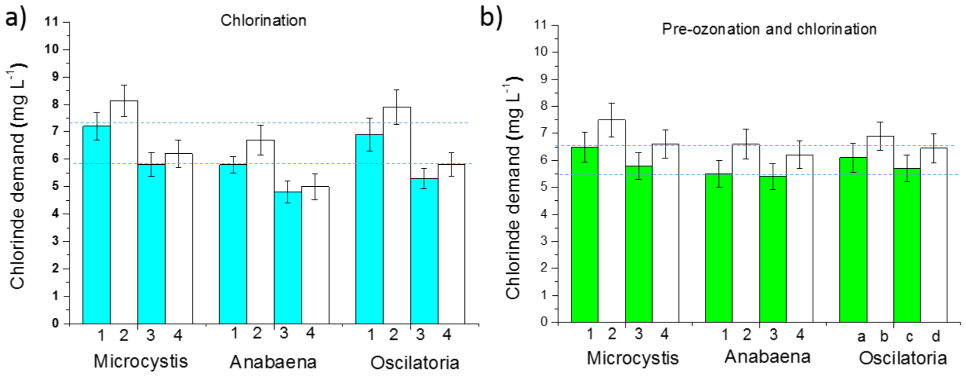

Figure 2. Chlorine demand of cyanobacteria aqueous suspensions in the presence and in the absence of bromide ions at pH 8 or 6.5. Legend of left figure, a): Chlorination at pH 8 in the absence (1) or the presence (2) bromide ions; Chlorination at pH 6.5 in the absence (3) or the presence (4) bromide ions. Legend of right part, b): Pre-ozonation followed by chlorination at pH 8 in the absence (1) or the presence (2) bromide ions; Pre-ozonation at pH 8 followed by chlorination at pH 6.5 in the absence (3) or the presence (4) bromide ions. General reaction conditions: Cyanobacteria concentration (10,000 cells/mL), ozone dose (1.6 mg/L), chlorine dose (11 mg/L), pH as indicated, 20 ºC, 72 h reaction time.

The presence of bromide ions at concentrations typically found in ground waters (300 μg L^{-1}) either at pH 8 or 6.5 increases the chlorine demand for the three cyanobacteria (10,000 cells/mL) under study. In the presence of bromide, the chlorine demand is also higher at pH 8 than at pH 6.5. This increase of the chlorine demand when Br^- is present can be explained considering that chlorine in the form of HClO or ClO^-

oxidizes Br^- to HBrO or BrO^-. The oxidizing character of HBrO (E_{RED}^0 = 1.33 eV) or BrO^- (E_{RED}^0 = 0.761 eV) is slightly lower respect to their respective chlorine analogous. In contrast, the reactivity of bromine respect to chlorine is higher towards electrophilic aromatic substitution. This fact is due to the higher carbocation stability substituted by bromide atoms that enjoys higher electron density and smaller bond strength compare with the chlorine ones(Deborde and von Gunten 2008). The higher chlorine demand in the presence of bromide ions or at pH 8 will be mainly reflected in an increase of THMs and HAAs (see below). In contrast, HANs are formed preferentially at acidic pH values due to their partial instability at basic pH values in which these DBP become hydrolyzed to haloacetamides. Thus, the higher chlorine demand accompanied by higher DBP formation, generally observed during water disinfection, also applies for the case of cyanobacteria chlorination in the presence of bromide ions.

For comparison, the chlorine demand of Microcystys aurea in the presence of bromide was also measured at high cyanobacteria concentration (1,000,000 cells/mL) at pH 8. A chlorine demand value of is 17.2 mg L^{-1} was determined. This chlorine demand when referred to the TOC of the cyanobacteria suspension (2.3 mg/L TOC) is somewhat lower (7.5 mg Cl_2/mg TOC) than that measured for a concentration of 10,000 cells/mL (8.1 mg Cl_2/mg TOC). This deviation may be attributed to the agglomeration of the cyanobacteria at higher concentration in the aqueous suspension and, therefore, decreasing slight chlorine accessibility to biomolecules. As it will be commented latter, the higher cyanobacteria concentration, the higher DBP production in absolute values (µg/L), but the lower chlorine demand referred to the TOC is reflected also in the relatively lower DBP production (µg/mg of TOC).

3.2. Influence of cyanobacteria chlorination and pre-ozonation on DBP formation

This section addresses the influence that the presence of the three cyanobacteria (10,000 cells/mL) under study in water on the DBP formation (THMs, HAAs, HANs, TCNM and HKs) upon chlorination with or without pre-ozonation. The study has been carried out at pH values of 8 or 6.5 in the presence or absence of bromide ions. Some of the results are presented in Figures 3-6 and Tables S1-S3. Figures 3-5 collects the THM and HAA formation after 1 and 3 days of chlorination. HAN formation is presented after 3 days of chlorination since the values 1 day after chlorination are below the quantification limit (0.5 μg L^{-1}). HK formation has not been included in the plots since in all the experiments its value is below the quantification limit (1 μg L^{-1}). TCNM was not observed in the chlorination, but it has been quantified when a pre-ozonation process is performed in the absence of bromide ions (Figure 6).

In general, the chlorination of the three cyanobacteria under study results in the formation of THMs and HAAs as the main C-DBPs. It was observed that DBP formation for Microcystis aeruginosa is always somewhat higher than for the other two cyanobacteria under study. This fact follows the higher chlorine demand of Microcystis aeruginosa and the higher protein content respect to Anabaena aequatis or Oscillatoria tenuis. HAN concentrations have been found to be below 5 μg/L.

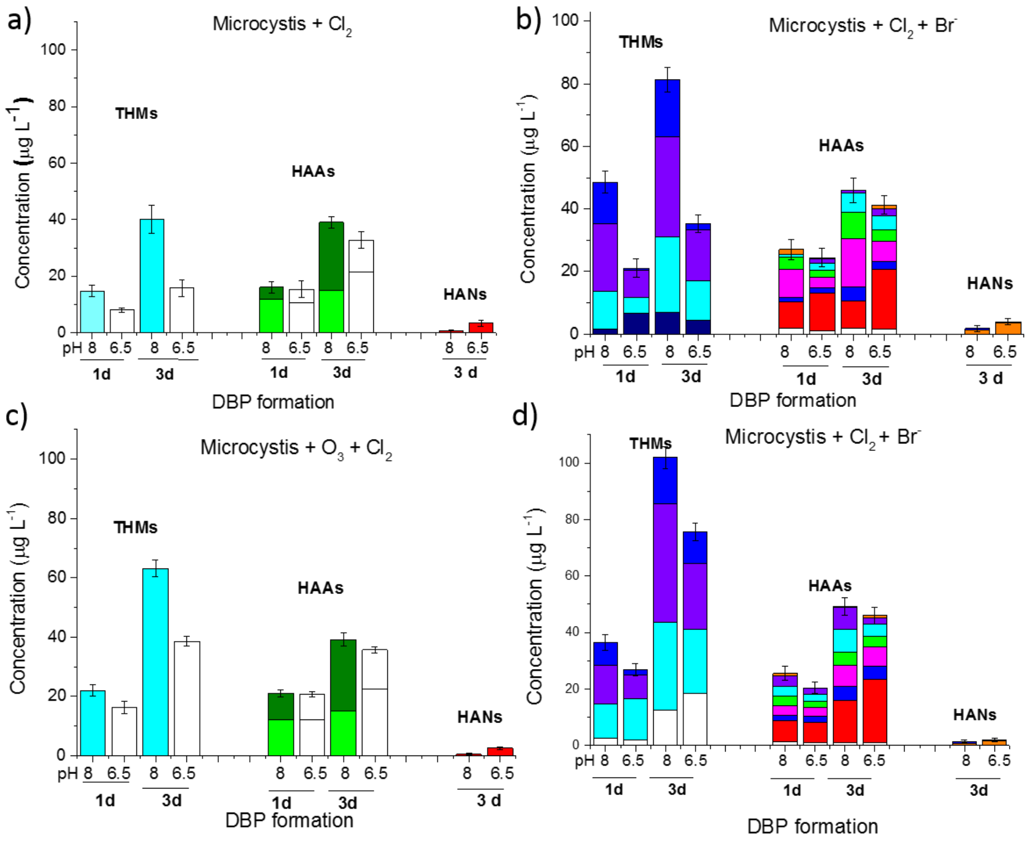

Figure 3. DPB formation after one (1 d) or three (3 d) days for Microcystis aqueous suspensions under chlorination without (a, b) or after pre-ozonation (c, d) as a function of the pH and in the absence (a, c) or presence (b, d) of bromide ions. Legend panels a and c: THMs corresponds to TCM, HAAs correspond to DCAA (bottom part) and TCAA (upper part), HANs correspond to DCAN. Legend panels b and d: THMs (TCM-white, BDCM-cyan, CDBM-purple, TBM-blue), HAAs (MCAA-yellow, MBAA-white, DCAA-red, TCAA-blue, BCAA-pink, BDCAA-green, DBAA-cyan, CDBAA-purple, TBAA-orange), HANs (DCAN-orange, TCAN-blue). Reaction conditions: Cyanobacteria concentration (10,000 cells/mL), chlorine dose (11 mg/L), ozone dose (1.6 mg/L), pH as indicated, bromide ions (300 µg/L), temperature (20 ºC).

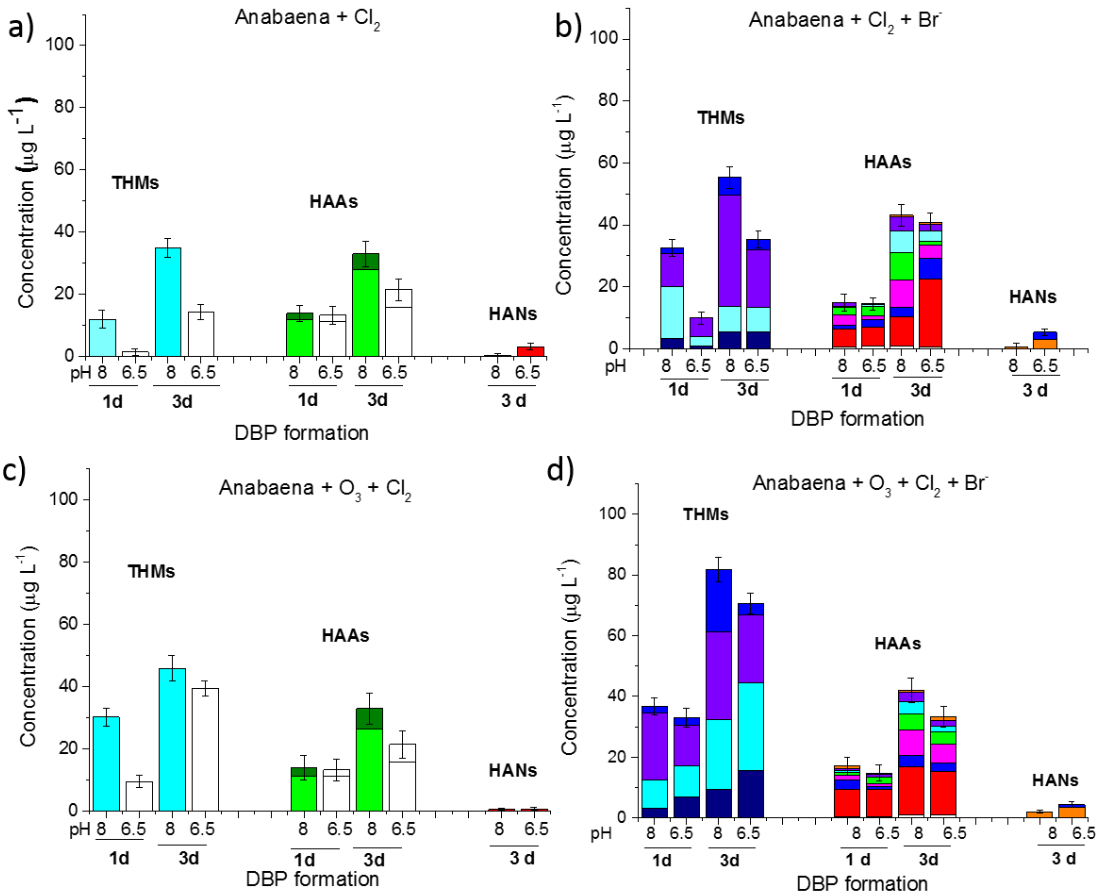

Figure 4. DPB formation after one (1 d) or three (3 d) days for Anabaena aqueous suspensions under chlorination without (a, b) or after pre-ozonation (c, d) as a function of the pH and in the absence (a, c) or presence (b, d) of bromide ions. Legend panels a and c: THMs corresponds to TCM, HAAs correspond to DCAA (bottom part) and TCAA (upper part), HANs correspond to DCAN. Legend panels b and d: THMs (TCM-white, BDCM-cyan, CDBM-purple, TBM-blue), HAAs (MCAA-yellow, MBAA-white, DCAA-red, TCAA-blue, BCAA-pink, BDCAA-green, DBAA-cyan, CDBAA-purple, TBAA-orange), HANs (DCAN-orange, TCAN-blue). Reaction conditions: Cyanobacteria concentration (10,000 cells/mL), chlorine dose (11 mg/L), ozone dose (1.6 mg/L), pH as indicated, bromide ions (300 μg/L), temperature (20 ºC).

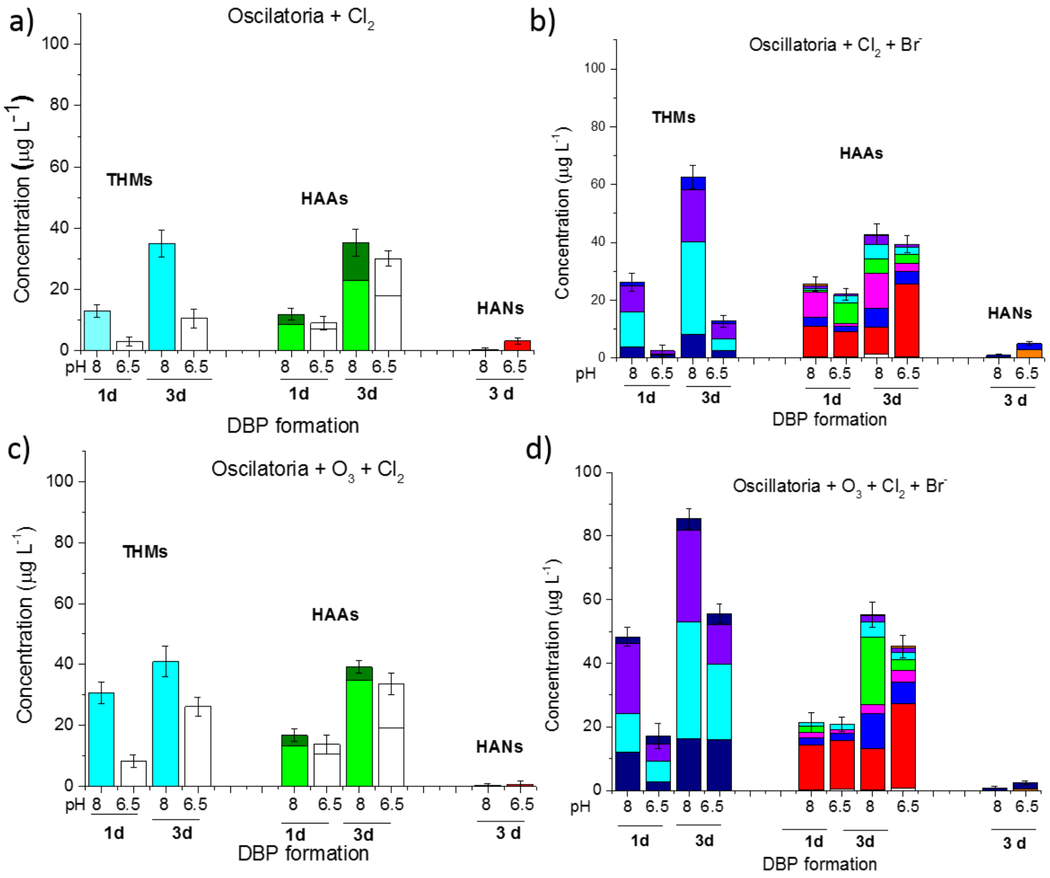

Figure 5. DPB formation after one (1 d) or three (3 d) days for Oscillatoria aqueous suspensions under chlorination without (a, b) or after pre-ozonation (c, d) as a function of the pH and in the absence (a, c) or presence (b, d) of bromide ions. Legend panels a and c: THMs corresponds to TCM, HAAs correspond to DCAA (bottom part) and TCAA (upper part), HANs correspond to DCAN. Legend panels b and d: THMs (TCM-white, BDCM-cyan, CDBM-purple, TBM-blue), HAAs (MCAA-yellow, MBAA-white, DCAA-red, TCAA-blue, BCAA-pink, BDCAA-green, DBAA-cyan, CDBAA-purple, TBAA-orange), HANs (DCAN-orange, TCAN-blue). Reaction conditions: Cyanobacteria concentration (10,000 cells/mL), chlorine dose (11 mg/L), ozone dose (1.6 mg/L), pH as indicated, bromide ions (300 μg/L), temperature (20 ºC).

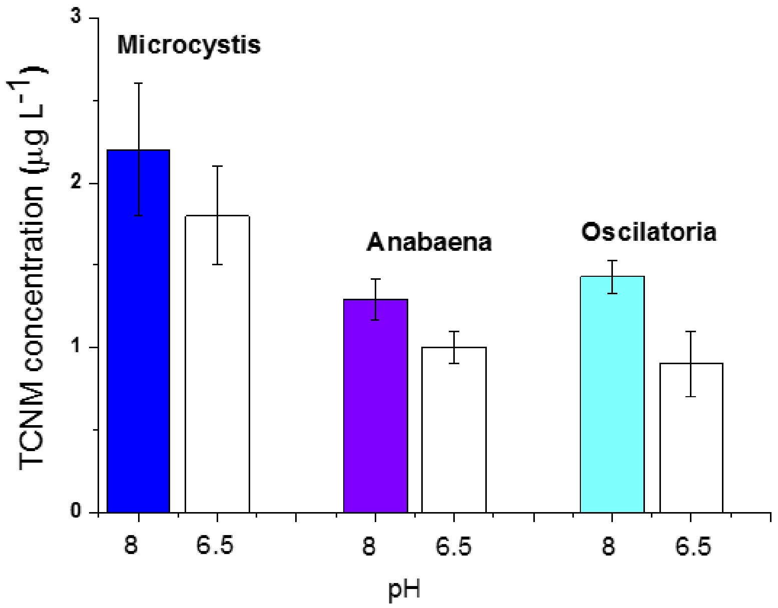

Figure 6. TCNM formation after 3 days upon pre-ozonation followed by chlorination of the cyanobacteria suspensions as a function of the pH of the aqueous solution. Reaction conditions: Cyanobacteria concentration (10,000 cells/mL), chlorine dose (11 mg/L), ozone dose (1.6 mg/L), pH as indicated, temperature (20 ºC).

3.2.1. DBP formation as a function of the pH of the chlorination

Figures 3-6 show the formation of THMs, HAAs and HANs after chlorination of cyanobacteria aqueous suspensions of Microcystis aeruginosa, Anabaena aequalis and Oscillatoria tenuis upon chlorination at pH 8 or 6.5 during 24 or 72 h. Obviously, exclusive formation of chlorinated DBPs is observed when the chlorination process occurs in the absence of bromide ions. Regardless the slightly higher DBP formation when using Microcystis aeruginosa some general comments can be drawn for the three cyanobacteria under study.

The main DBPs formed upon chlorination of cyanobacteria suspensions at pH 8 are THMs and HAAs, reaching concentrations of 35±0.12 and 29±3.6 μg/L, respectively, at 72 h. The only THM observed is chloroform, while the HAAs are mainly constituted by DCAA and TCAA. In addition, THM and HAA concentrations increase over the time, an observation that is in agreement with the stability of these compounds in aqueous solutions under ambient conditions and quasi-neutral pH values. In the case HANs, DCAN is the main DBP observed with concentration values for the three cyanobacteria at pH 8 of about 0.97±0.55 μg L^{-1} after 72 h. Previous studies have shown that chlorination of free amino acids leads to the formation of chloroform, DCAN and trichloroacetaldehyde as main DBPs(Trehy et al. 1986). The absence of TCAN was attributed to its high hydrolysis rate(Bond et al. 2011, Glezer et al. 1999).

As commented before, a decrease of the pH of the solution from 8 to 6.5 results in a general decrease about 15 % of the chlorine demand. The influence of the pH during the chlorination is, however, strongly dependent on the DBP nature. When the pH decreases from 8 to 6.5, the THM and HAA concentrations are reduced about 50 and 20 %, respectively.

Regarding HAN formation upon cyanobacteria suspension chlorination, its concentration increases as the pH decreases for the three cyanobacteria from 0.97±0.55 μg L^{-1} to 3.3 μg L^{-1} after 72 h. This observation agrees with previous reports that observed a maximum HAN stability in water at pH 6.5, while an increase of the pH decreases the HAN concentration due to their hydrolysis to their corresponding haloacetamides(Chu et al. 2015, Glezer et al. 1999)). Haloacetamides can be further hydrolyzed to their corresponding HAAs(Glezer et al. 1999).

3.2.2. Effect of the presence of bromide ions on DBP formation during chlorination of the cyanobacteria as a function of the pH

In addition to the influence of the pH on DBP formation, the presence of bromide ions is also an important factor. The general trend observed during water chlorination is that the presence of bromide ions, even at microgram per liter concentrations, increases the total DBP formation and results in the formation of brominated DBPs. Figures 3-5 summarize the results for the DBP formation from the three cyanobacteria under study (10,000 cells/mL) in the presence of bromide ions at 300 μg L^{-1} at both pH 8 or 6.5. As it can be seen there, the presence of bromide ions increases the concentration of THMs at both pH 8 and 6.5 reaching average values of 68.7±11.9 and 26.9±14.5 μg L^{-1}, respectively. In the case of HAAs, the presence of bromide ions mainly favors the formation of brominated HAAs with slight increase of the total concentration at both pH 8 and 6.5 with average values of 60.7±5.0 and 40.2±1.6 μg L^{-1}, respectively. In the case of HANs the presence of bromide ions also increases the total HAN concentration, reaching values of 1.2±0.6 and 4.8±0.7 μg L^{-1} at pH 8 and 6.5, respectively, while favoring the formation of brominated HANs.

Figure 7 shows the bromide incorporated into THMs and HAAs during the chlorination of the three cyanobacteria under study in the presence of bromide ions at pH 8 and 6.5. As it can be seen there, the proportion of brominated THMs is between 90 and 70 % a fact that agrees with the higher stability of brominated methyl anions (CBr_3^-) than chlorinated (CCl_3^-) ones (Navalon et al. 2008). In the case of HAAs the proportion of brominated compounds is lower than in the case of THMs especially at pH 6.5. It should be reminded that since HClO pKa is 7.54, it prevails at pH 6.5 in the acid form. Considering that HClO is a stronger oxidant than ClO^-, more oxidized and chlorinated compounds such as DCAA can be expected, as in the present case. This situation, i.e. the lower bromide incorporation at pH 6.5 respect to pH 8, although in less extent, also applies for THMs.

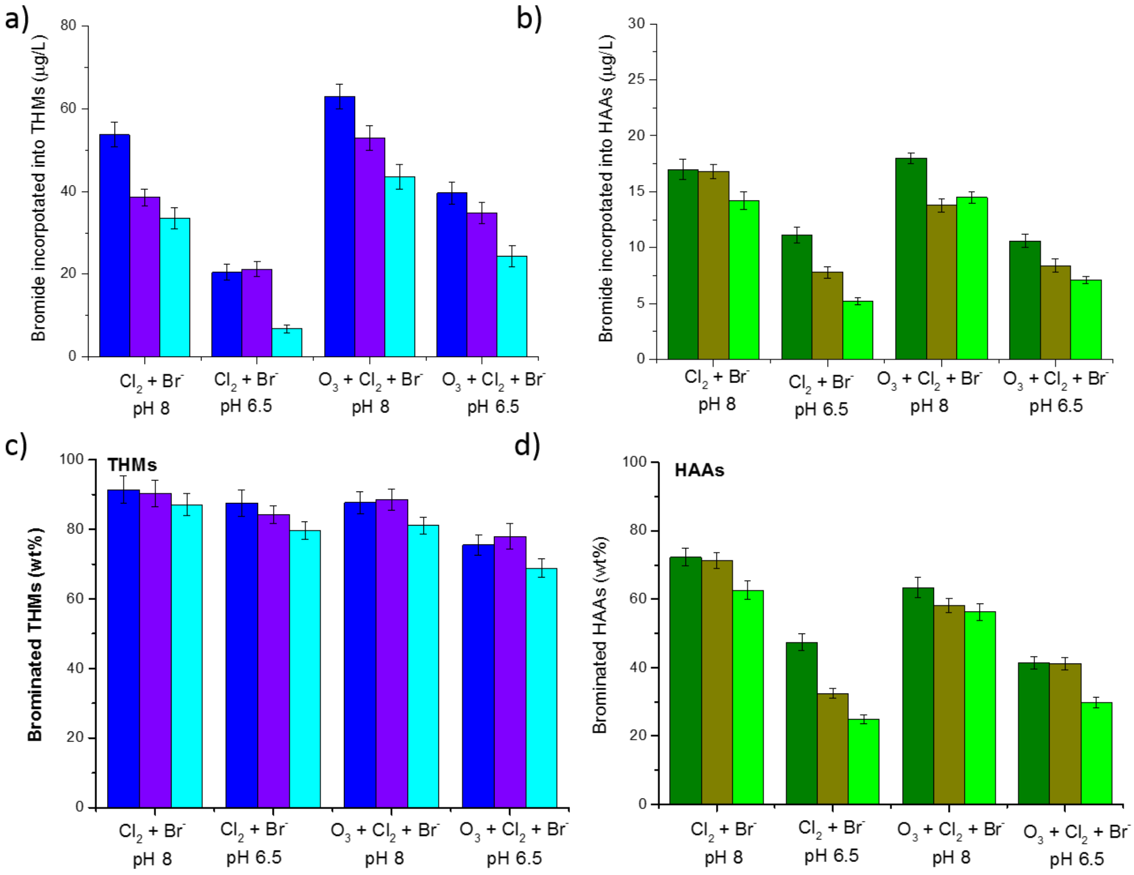

Figure 7. Bromide incorporation into THMs (a) or HAAs (b) and percentage of brominated THMs (c) or HAAs (d) during the chlorination of Microcystis aeruginosa (black bar), Anabaena aequalis (red bar) and Oscillatoria tenuis (blue bar) cyanobacteria under various reaction conditions as indicated. Cyanobacteria concentration (10,000 cells/mL), chlorine dose (11 mg/L), ozone dose (1.6 mg/L), pH as indicated, bromide ions (300 µg/L), temperature (20 ºC).

When considering the possible differences in DBP distribution among the three cyanobacteria under study as presented in Figure 7, it has to be considered that assuming a normal Gaussian distribution, the 95.5% level of confidence for the lower and higher limit for a value corresponds to two times the standard deviation (indicated for each bar). Therefore, there are certain data (as THM values shown in frame c of Figure 7) that correspond to essentially identical statistical values, while there are others, like formation of $CHCl_2Br$ in Oscillatoria tenuis that is certainly below the values formed for the other two cyanobacteria.

In the case of Microcystis an additional chlorination was carried out at a cyanobacteria concentration of 1,000,000 cells/mL in the presence of bromide (300 μg/L). Formation of THMs, HAAs and HANs of about 143, 87 and 1.9 μg/L, respectively, (Figure S1) was measured. Again, the formation of TCNM or HKs was below the quantification limit.

3.2.3. Effect of pre-ozonation on DBP formation

The influence of pre-ozonation on DBP formation was studied at an ozone dose of 1.6 mg/L. This ozone dose is in the average range typically employed in water treatment plants. The use of higher ozone dose can compromise the possibility of using ozone to control DBPs, due to the oxidation of the naturally occurring bromides to bromates. Bromate concentration is limited by most drinking water regulations to the value of 10 μg/L(von Gunten 2003). In this work, bromate concentrations after ozonation have been always found below 4 ± 0.7 μg/L. Moreover, a pre-ozonation of the cyanobacteria aqueous suspensions under study decreases the chlorine demand respect to the chlorination process (Figure 2b). However, this chlorine demand decrease does not diminish the concentration of all the DBPs considered in this study (THMs, HAAs, HANs, TCNM or HKs) and in some cases these concentrations even increase significantly. It should be noted that DBP formation occurs in the range of about 0.1 mg/L, while chlorine consumption is one order of magnitude higher in the range of about 6 mg/L.

More specifically, a pre-ozonation followed by chlorination of the three cyanobacteria under study results in an increase of THM formation both at pH 8 or 6.5 either with or without bromide ions up to 50 % respect to the chlorination process (Figures 3-5). In the case of HAA formation from the three cyanobacteria also at pH 8 or 6.5 in the presence of absence of bromide ions, the pre-ozonation process does not significantly diminish and even sometimes slightly increases its concentration. With these precedents, our work further exemplifies that a pre-ozonation at doses commonly employed in the water treatment plants of the three cyanobacteria under study working at relatively low cell concentrations (10,000 cells/mL) working at either pH 8 or 6.5 with or without bromide ions results in a significant increase of THMs and poor control of HAA concentration that in sometimes increases.

The reasons for the observed increase of THMs and HAAs due to a pre-ozonation of cyanobacteria aqueous suspensions still remain unknown. On one hand, there is limited knowledge on how ozonolysis transforms complex biomolecules such as those present in cyanobacteria(Sharma and Graham 2010, von Gunten 2003). On the second hand, ozonation reaction conditions can determine the contribution of ozonolysis vs. radical mechanism with the generation of hydroxyl radicals(von Gunten 2003). Besides electrophilic attack of ozone, this molecule can decompose in water, especially at basic pH values, into reactive oxygen species, such as hydroxyl radicals, that can virtually react non-selectively with any organic compound. Thus, further studies about the reactivity of ozone with natural complex biomolecules are necessary to understand the effect of the pre-ozonation of cyanobacteria on THM and HAA formation.

In this context, some studies have reported the release of free amino acids upon oxidation of peptides and proteins by hydroxyl radicals and this release should influence DBP formation(Liu et al. 2017). For instance, regarding peptide reactivity, it has been reported that free tyrosine (Tyr) forms 74 % more chloroform than Tyr-Tyr-Tyr peptide(Chu et al. 2015). The same study also found different chlorine reactivity towards THM formation of NH_2- or COOH-terminated peptide isomers such as tyrosine-alanine or alanine-tyrosine(Chu et al. 2015). Other study has reported that preoxidation of the amino acids leucine and serine by a UV/H_2O_2 treatment followed by chlorination results in an increase of HAA formation(Sakai et al. 2013). In this sense, the increase of HAA formation of pre-ozonized cyanobacteria may be partially attributed to similar reaction pathways. These results exemplify the complexity of chlorine reactivity for peptides.

Similarly to peptides, the reactivity of ozone with polysaccharides and the consequences for DBP formation are difficult to predict(Sharma and Graham 2010). Only few studies have reported the production of THMs from mono- and oligosaccharides. It seems that oligosaccharides such as maltopentose or maltotriose produce higher THM amounts than the monosaccharide maltose(Navalon et al. 2008). Other studies have reported that ozonolysis is able to depolymerize polysaccharides containing β-D-aldosidic linkages(Wang et al. 1998). As in the case of proteins, it is not possible to predict the effect of pre-ozonation on THM formation in polysaccharides.

Figure 7 shows that pre-ozonation increases significantly brominated THMs at pH 8 without changing significantly the percentage of Br-DBP respect to the THM total. Some reports using humic acids as DBP precursors have shown that pre-ozonation results in a shift of DBPs towards brominated ones(Mao et al. 2014), but this is not the case of pre-ozonation of the aqueous suspensions of the three cyanobacteria (Figure 7c,d).

Regarding N-DBPs, while TCNM is not observed either at pH 8 or 6.5 when the aqueous suspensions of the three cyanobacteria were submitted to a chlorination, its presence could be quantified after pre-ozonation and chlorination of the samples in the absence of bromide ions (Figure 6). In agreement with previous works(Hu et al. 2010), TCNM formation is favoured at basic pH values. The fact that TCNM is again not observed when pre-ozonation is carried out in the presence of bromide ions is due to the formation of other N-DBPs related to TCNM, but containing bromide replacing to chlorine atoms(Hu et al. 2010).

A reasonable pathway to understand the formation of TCNM upon pre-ozonation considers the release of free amino acids due to oxidation of peptides and proteins by hydroxyl radicals(Liu et al. 2017), followed by decarboxylation of the amino acid and subsequent halogenation of the resulting amines(Le Lacheur and Glaze 1996). In this regard, it has been reported that ozone promotes TCNM formation by oxidizing amines to nitro compounds that subsequently undergo chlorination to halo nitro compounds (Figure 8b)(McCurry et al. 2016). This effect has also been reported when using natural waters(Bond et al. 2011). The higher TCNM formation at basic pH values would be due to the easier oxidation of deprotonated amino groups to nitro groups. In addition, basic pH values favor the partial O_3 decomposition to hydroxyl radicals that are better oxidizing reagents promoting the conversion of amino to nitro groups.

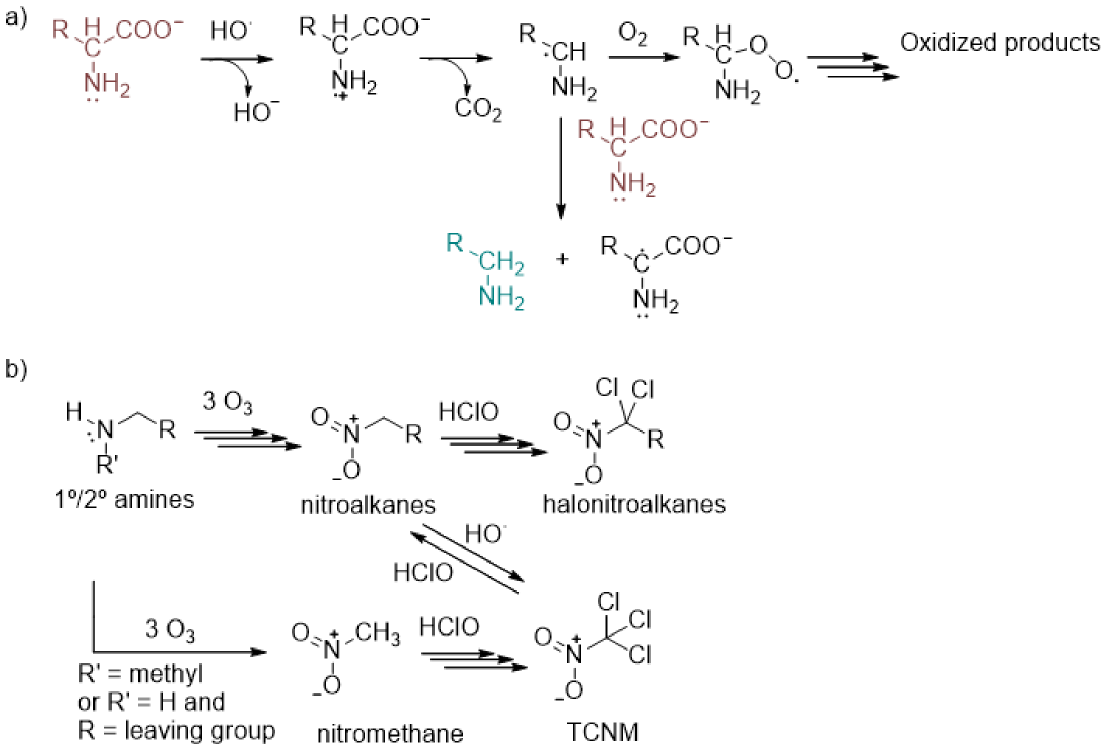

Figure 8. Proposed reaction pathway for generation of halonitroalkanes upon pre-ozonation and chlorination of amino acids and amines. Adapted from ref. (McCurry et al. 2016)

In accordance to the mechanism leading to TCNM, formation of this DBP after pre-ozonation would occur at the expense of a decrease in the concentration of HANs. Oxidation of the α-carbon to the nitrile by ozone would hamper0 its chlorination.

4. Conclusions

The influence of the presence of three common cyanobacteria commonly encountered in fresh waters, namely Microcystis aeruginosa, Anabaena aequalis and Oscillatoria tenuis at relatively low cell concentration (10,000 cells/mL) on DBP formation depending on the pH and pre-ozonation has been studied. Cyanobacteria chlorination results in the formation THMs and HAAs as main C-DBPs, accompanied by minor amounts of N-DBPs such as HANs and absence of HKs and TCNM. Basic pH values increases formation of THM and in less extent HAAs, decreasing HAN concentration. The presence of bromide ions increases THM and HAN concentrations, while the formation of HAAs is promoted in less extent.

Pre-ozonation (1.6 mg/L O_3) of the aqueous suspensions containing the three cyanobacteria under study was carried out under conditions in which bromate formation (4 ± 0.7 μg/L) is below the maximum legal concentration (10 μg/L). Pre-ozonation increases THM formation and generates TCNM, while has minor influence on HAA formation. In contrast, this oxidation process decreases HAN formation.

In summary, the present work shows that common cyanobacteria present in fresh waters are important DBP precursors and pre-ozonation is not a general method to decrease the main C- and N-DPBs.

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