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Disinfection byproduct formation from chlorination of pure bacterial cells and pipeline biofilms

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ABSTRACT

Disinfection byproduct (DBP) formation is commonly attributed to the reaction between natural organic matters and disinfectants, yet few have considered the contribution from disinfecting bacterial materials – the essential process of water disinfection. Here, we explored the DBP formation from chlorination and chloramination of *Escherichia coli* and found that most selected DBPs were detectable, including trihalomethanes, haloacetonitriles, chloral hydrate, chloropicrin, and 1,1,1-trichloro-2-propanone. A positive correlation ($P = 0.08–0.09$) between DBP formation and the log reduction of *E. coli* implied that breaking down of bacterial cells released precursors for DBP formation. As *Pseudomonas aeruginosa* is a dominant bacterial species in pipeline biofilms, the DBP formation potentials (DBPFPs) from its planktonic cells and biofilms were characterized. Planktonic cells formed 7–11 times greater trihalomethanes per carbon of those from biofilms but significantly lower ($P < 0.05$) chloral hydrate, highlighting the bacterial phenotype's impact on the bacteria-derived DBPFP. Pipe material appeared to affect the DBPFP of bacteria, with 4–28% lower bromine incorporation factor for biofilms on polyvinyl chloride compared to that on galvanized zinc. This study revealed both the *in situ* disinfection of bacterial planktonic cells in source water and *ex situ* reaction between biofilms and residual chlorine in pipeline networks as hitherto unknown DBP sources in drinking water.

1. Introduction

Water disinfection is one of the most important public health advances of 20th century that significantly reduces the waterborne pathogen-induced diseases. Since chloroform was first discovered as disinfection byproduct (DBP) during water chlorination in 1974 (Rook, 1974), increasing evidences on health risks studies (Boorman et al., 1999; Bove et al., 2002; Richardson et al., 2007, 2010) indicated that the DBPs (such as trihalomethanes (THMs), haloacetic acids (HAAs), haloacetonitriles (HANs) and chloral hydrate (CHD)) were potential carcinogens, teratogens or mutagens. To water engineers and environmental microbiologists, it is a great challenge to balance the risk between microbial and DBP contaminations for safe drinking water. Numerous studies have been conducted to understand the DBP formation in drinking waters,

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but their focuses are on reactions between disinfectants and litter leachates or soil runoffs (particularly humic substances) in source water (Reckhow et al., 1990; Singer, 1999; Chow et al., 2005, 2009; Pellerin et al., 2010). The disinfection process is a series of reactions between disinfectants and pathogens/microorganisms, yet few studies have evaluated the DBP formation from the microbiological point of view and correlated the DBP formation with bacterial disinfection.

Breaking down of bacterial cells, resulting in releases of dissolved organic materials like polysaccharides, proteins, and nucleic acids, is an essential process in water disinfection (Zhang et al., 2010). The bacteria-derived organic matters are generally enriched in nitrogen and have smaller molecular weight than humic substances, and therefore their reactivity in forming DBPs and species should be different. Importantly, it is frequently reported that bacterial contamination occurred in source waters (Davis et al., 2005; Jenkins et al., 2005; Ruecker et al., 2007) and water distribution systems (Langmark et al., 2005; Batte et al., 2006; September et al., 2007), and thus the bacterial materials may contribute DBP formation in finished waters. Previous reports (Hong et al., 2008; Navalon et al., 2008; Wang and Zhang, 2010; Wei et al., 2011) suggested that the biomolecules can serve as the DBP precursors. Also, our research team found that some pure bacterial strains produced 6.1–37.6 μg-THMs/mg-C, 3.2–16.3 μg-HANs/mg-C and 0.65–1.98 μg-CHD/mg-C during the reaction with NaOCl solution (Wang et al., 2012), and speculated that the bacteria could be important DBP precursors in water treatment. However, little is known on how and to what extent the various DBPs are formed from bacterial materials by different disinfection processes or disinfecting doses, and their relationships with the efficacy of bacterial disinfection.

Along the drinking water supply facilities, the bacteria from multiple sources dominate in different phenotypes (planktonic cells vs. biofilms). Planktonic cells are ubiquitous in the untreated source water from watershed (Davis et al., 2005; Jenkins et al., 2005; Ruecker et al., 2007), whereas in the water distribution networks, biofilms are often formed on the pipeline walls (Langmark et al., 2005; Batte et al., 2006; September et al., 2007). Both phenotypes can grow fast by turning large mass of plant-derived organic matter into bacterial materials and thus may change the DBP formation potential (DBPFP). However, even of the same bacterial species, the biofilm cells covered by self-produced matrix of extracellular polymeric substance (EPS) are physiologically distinct from planktonic cells that suspend in the water (Romeo, 2008; Flemming and Wingender, 2010). Their distinct biochemical compositions may lead to different contributions to the DBP formation, in terms of both DBP quality and quantity. Moreover, the pipe materials where biofilms grow on were shown to affect the biofilm cell growth (Kerr et al., 1999; Niquette et al., 2000; Hallam et al., 2001; Li and Zhang, 2003; Liu et al., 2007) but their impact on the bacterial carbon quality for DBP formation is still unknown.

In the present study, the most common bacteria *Escherichia coli* was selected to examine its DBP formation via different dosages of chlorination and chloramination processes and the corresponding bacterial survival (an indicator for disinfection efficacy). To explore the effects of growth modes and the surface materials of drinking water pipes on DBP formation from bacterial materials, the planktonic cells of *Pseudomonas aeruginosa* in water and the corresponding biofilms on either polyvinyl chloride (PVC) or galvanized zinc (GZ) materials were selected for the DBPFP tests.

2. Materials and methods

2.1. Chlorination and chloramination of E. coli

To examine and compare DBPs generated from chlorination and chloramination on pure bacteria within short time, the bacterium, *E. coli* K-12, was disinfected for 1 h using chlorine or chloramine. The bacterial cells were cultured in nutrient broth (NB, BioLife, Milano, Italy) at 37 °C and stirred on a rotary shaker at 200 rpm for 16 h to reach the stationary phase (10^9 colony forming unit (cfu)/mL). The cultures were then washed three times with sterilized 0.9% NaCl (saline) solution (>99.5%; Sigma–Aldrich) and centrifuged at 11,600 g for 5 min to isolate the cells from the broth. Ultrapure water, pipette tips, and all glassware used were all autoclaved (121 °C for 20 min) to avoid culture contamination.

Freshly prepared saline-washed bacterial cell was resuspended in 65 mL freshly prepared sterilized saline solution (in which bacterial carbon was adjusted to 2.0 mg/L) with NaOCl (0, 1, 2, and 3 mg-Cl/L) or monochloramine (NH_2Cl) (0, 0.5, 1.0, and 2.5 mg/L) without headspace. A series of NaOCl solutions were diluted from 1000 mg-Cl/L solution (Aldrich) for chlorination. For chloramination, all diluted NH_2Cl solutions were from freshly prepared 100 mg/L NH_2Cl solution, which was made by slowly adding the NaOCl solution to NH_4Cl solution (Sigma) (mass Cl/N ratio = 3:1; stirring for 1 h) with pH adjusted to ~8.5. Both chlorination and chloramination reactions were controlled within pH of 8.0 ± 0.2 by using borate buffer. These reactions were stopped at five different time intervals (0, 10, 20, 40, and 60 min) using quenching solutions (200 μL of 5% NH_4Cl for chlorination; 10 μL of 10% Na_2SO_3 for chloramine treatment). After reaction, the treated bacterial solution was immediately spread on nutrient agar (Lab M Limited) plate and incubated at 30 °C for 24 h to determine the numbers of viable cell. The DBPs generated after 1 h reactions were extracted by methyl tert-butyl ether and quantified by GC-ECD.

2.2. Biofilm incubation and disinfection

To evaluate the DBP formation from pipeline biofilm, *P. aeruginosa* (PAO1) was chosen because it is a commonly dominant species in tap water pipe wall biofilms (Schwartz et al., 1998; Lee and Kim, 2003; Martiny et al., 2003; Moritz et al., 2010). The bacterial cells were cultured in nutrient broth solution at 30 °C for 18 h with agitation of 200 rpm to a stationary phase (10^9 cfu/mL). The culture was then washed with saline solution and centrifuged for 5 min, after which the cell pellet was resuspended in saline solution. The cell density was adjusted to 2-3 × 10^6 cfu/mL to be subsequently incubated.

Polyvinyl chloride (PVC) and galvanized zinc (GZ) materials were chosen as media for biofilm formation because of their common application as pipeline materials (Niquette et al., 2000). The materials were processed into 10 mm × 10 mm × 1 mm chips, cleaned with 95% ethanol and then ultrapure water, and

then autoclaved at 120 °C. To determine the dynamics of biofilm formation, three 65 mL sterile glass vials, each containing 6 pieces of chips, 100 μL bacterial solution and 20 mL NB solution for both PVC and GZ, were incubated at 25 °C in the dark. After 4, 8, 12, 24, 36 and 60 h, one chip was taken out from each vial and gently washed with saline solution to remove the loosely attached bacteria. Sessile *P. aeruginosa* was scraped repeatedly by a sterilized cotton swab which was then transferred into 2 ml saline solution and ultra-sonicated for 15 min. An appropriate dilution of the detached sample was then immediately spread onto nutrient agar plates and incubated at 37 °C for 24 h. The number of colonies formed was counted to determine the cell density of the biofilm. For the DBPFP evaluation, 50 pipe chips of each material were added into freshly prepared bacteria-containing NB solution (bacterial solution to NB solution = 1:200 on volume basis), for static incubation at 25 °C in the dark for 24 h. After incubation, 50 biofilm samples were separated from chips as aforementioned, 10 of which were then used for total organic carbon (TOC) determination and 40 for DBPFP analyses after chlorination based on the uniform formation conditions (UFCs) (Summers et al., 1996). To compare the difference of DBPFPs between biofilm and cell solution of *P. aeruginosa*, the fresh bacterial cell solution of *P. aeruginosa* (bacterial carbon = 1 mg/L) were also prepared for DBPFP analysis.

UFCs were adopted for the DBPFP assessment with sodium hypochlorite solution (Sigma–Aldrich) as chlorination reagent. Briefly, the bacterial suspension and biofilms of *P. aeruginosa* were chlorinated under the following conditions: 1) pH: 8.0 ± 0.2 adjusted by borate buffer; 2) temperature: 20.0 ± 1.0 °C; 3) incubation time: 24 ± 1 h; and 4) Cl residual: 1.0 ± 0.4 mg/L. All glassware used was pre-soaked in chlorine solution (20 mg/L), rinsed three times with tap water and then ultrapure water, and furnace heated (140 °C overnight) to remove chlorine and organic carbon according to the standard method (Eaton and Franson, 2005). All TOC was measured by combustion catalytic oxidation using a Shimadzu TOC-VCSH/CSN analyzer equipped with a solid sample measurement module SSM-5000A. The effect of bromide (Br^-) on DBP formation was also evaluated by adding Br^- (KBr) of different concentration (0, 0.5, 1.0, and 2.0 mg-Br^-/L) into the reaction system before the chlorination. We also calculated the bromine incorporation factor (BIF, the proportion of total halogen positions with bromine-substituted atoms) of THMs (Hua et al., 2006; Diaz et al., 2008; Hua and Reckhow, 2012), the largest and representative group of DBPs, so as to evaluate the abundance of organic bromine:

$$\mathrm{BIF} = \frac{\sum_{n=0}^{3} n \times [\mathrm{CHCl}_{3-n}\mathrm{Br}_n]}{3 \times \sum_{n=0}^{3} [\mathrm{CHCl}_{3-n}\mathrm{Br}_n]} \times 100\% \quad (1)$$

2.3. DBP quantification

After incubation, all samples were treated with 1–2 drops H_2SO_4 to stabilize DBPs from hydrolysis, and then immediately extracted by HPLC grade methyl tert-butyl ether (Fluka, Switzerland). Four trihalomethanes (THMs) (including trichloromethane (TCM), dichlorobromomethane (DCBM), dibromochloromethane (DBCM), and tribromomethane (TBM)), four haloacetonitriles (HANs) (including trichloro-acetonitrile (TCAN), dichloro-acetonitrile (DCAN), bromochloro-acetonitrile (BCAN), and dibromo-acetonitrile (DBAN)), chloral hydrate (CHD), chloropicrin (CHP), 1,1-dichloro-2-propanone (1,1-DCP) and 1,1,1-trichloro-2-propanone (1,1,1-TCP), were measured using GC-ECD (Agilent 6890) according to USEPA method 551.1. All DBP standards were purchased from Supelco, USA. The minimum reporting level (MRL) for all DBP species was approximately 1 μg/L.

3. Results and discussion

3.1. Bacterial survival and DBP formation

With 1–3 mg/L chlorine addition for more than 10 min, no *E. coli* survival was observed in the pure bacterial solution (originally 7.2 ± 0.2 lg(cfu)/mL) (Table 1). As a weaker disinfectant, chloramine gradually inactivated bacteria, with higher disinfection efficiency using high disinfection dose and longer contact time. When 2.5 mg/L chloramine was adopted, *E. coli* was effectively inactivated within 1 h though it recovered slightly to 1.6 ± 0.4 lg(cfu)/mL.

Although the reaction time was only 1 h, the bacterial carbon of *E. coli* (2.0 mg-C/L), i.e., the unique carbon source in the reaction system, generated substantial amount of DBPs after reaction with either chlorine or chloramine (Fig. 1). With increasing disinfecting reagent concentration, the total DBP yield was enhanced. Trihalomethanes were always the major DBPs increasing from 18.6 ± 1.5 μg/L using 1 mg/L chlorine to 35.5 ± 11.2 μg/L using 3 mg/L chlorine, and from 8.1 ± 1.1 μg/L using 0.5 mg/L chloramine to 12.3 ± 0.3 μg/L using 2.5 mg/L chloramine. Except for trichloromethane (all >6 μg/L), most of the individual chlorinated DBP species were below 6 μg/L and 1,1-DCP was not detectable. The mean total HAN concentration amounted to 11.5–12.2 μg/L after chlorination and 4.61–5.23 μg/L after chloramination dependent on the disinfecting dose. Chloropicrin (4.19–5.98 μg/L) and 1,1,1-TCP (4.07–5.66 μg/L) were both detected after the chlorination, but after chloramination chloropicrin was under detection limit and 1,1,1-TCP concentration ranged between 3.01 and 3.67 μg/L.

Combining the results of bacterial inactivation (Table 1) and DBP formation (Fig. 1) during chloramination, higher total DBP and THMs formation can be observed at higher log reduction of *E. coli* (higher disinfection efficacy) in the pure bacterial solution (Fig. 2), implying that breaking down of

Table 1 – Survival of *Escherichia coli* (bacterial count in lg(cfu)/mL) during chlorination and chloramination in 1 h (Mean ± SD from three replicates).

Time (min)	Control	Chlorine			Chloramine		
		1 mg/L	2 mg/L	3 mg/L	0.5 mg/L	1.0 mg/L	2.5 mg/L
0	7.2 ± 0.2						
10	7.0 ± 0.3	0	0	0	7.0 ± 0.0	5.4 ± 0.2	0
20	7.2 ± 0.2	0	0	0	6.1 ± 0.1	4.6 ± 0.4	0
40	7.4 ± 0.1	0	0	0	5.8 ± 0.1	4.1 ± 1.2	0
60	7.2 ± 0.1	0	0	0	4.4 ± 0.3	3.4 ± 0.2	1.6 ± 0.4

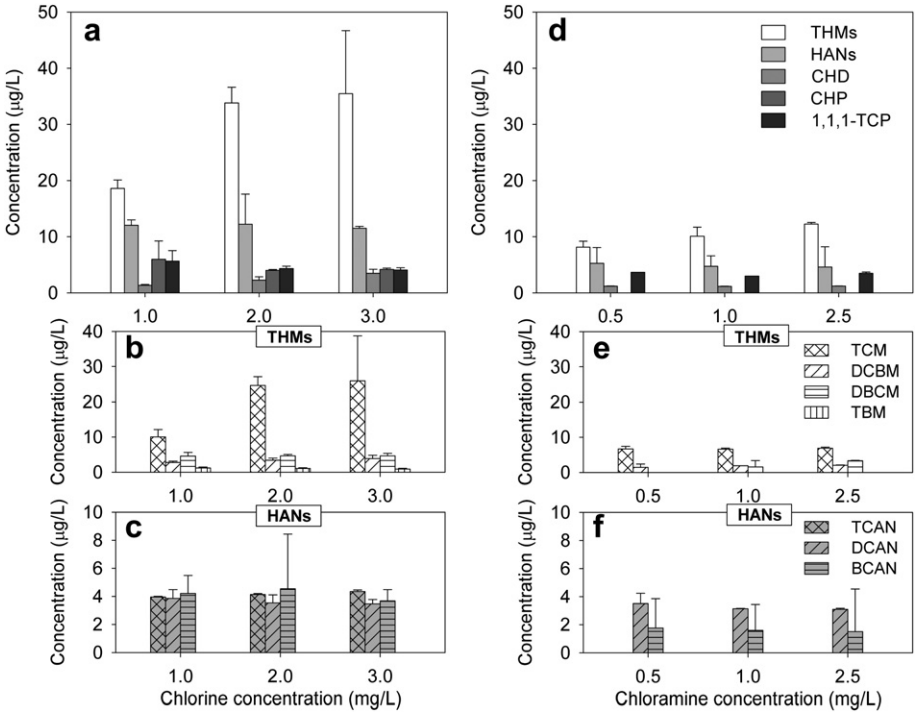

Fig. 1 – Disinfection byproduct formation after 1 h chlorination and chloramination of pure *Escherichia coli* solution. THMs, HANs and CHD refer to trihalomethanes, halo-acetonitriles and chloral hydrate, respectively. All error bars indicated the standard deviation of 12 replicates.

bacterial cells provides precursor for DBP formation. This evidence strongly supports the pathway for DBP formation from pure bacterial materials. As bacteria like *E. coli* are commonly ubiquitous in the natural raw water, and difficult to be physically removed, it is consequently not easy to avoid the DBPs generated from different bacterial disinfection processes.

The positive correlation between the log reduction of *E. coli* and DBP formation (Fig. 2) implies a tradeoff between the pathogen and DBP contamination in drinking water applying certain range of disinfectant dose. As DBP formation is nearly unavoidable to disinfect bacteria in source water, optimal technique and operation conditions are always desirable to balance and minimize both the pathogen and DBP risks for drinking water. In the present study, chloramination was likely to be a substitute for chlorination to reduce DBP formation in pure bacteria solution. Here, 46, 61 and 13% of THMs, HANs and CHD, respectively, could be reduced during the pure bacteria disinfection using 1 mg/L chloramine instead of 1 mg/L chlorine. In addition, within the DBP groups we studied, fewer types of DBP species were detectable when using chloramine as alternative.

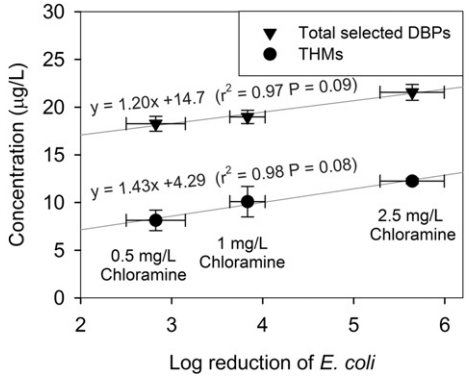

Fig. 2 – Correlations of total DBPs and trihalomethanes (THMs) formation with the log reduction of *E. coli* after 1 h chloramination. All error bars indicated the standard deviation of 12 replicates.

3.2. *DBP formation from pipe biofilm—distinct from planktonic cells*

3.2.1. *Biofilms formation on pipe materials*

After inoculated in the chip containing solution, bacteria *P. aeruginosa* attached on the surfaces of both two pipe materials and form biofilms rapidly, having no significant difference in biofilm growth between PVC or GZ surfaces (paired *t*-test, $n = 18$, $P < 0.001$; Fig. 3a). The biofilms both reached maturation after 24 h incubation and the viable cell number reached 6.5 ± 0.6 lg(cfu)/cm^2 on PVC surface and 6.5 ± 0.7 lg(cfu)/cm^2 on GZ surface. No significant differences (*t*-test, $n = 3$, $P < 0.01$) were observed for viable cell numbers at 24 h and those at 36 h, but the viable cell numbers at 60 h declined to 5.6 ± 0.2 lg(cfu)/cm^2 on PVC surface and 5.3 ± 0.6 lg(cfu)/cm^2 on GZ surface, suggesting that the biofilms reached to dispersion stage after 36 h. Because the inertness and rough surface of GZ material is in favor of the bacterial attachment and growth, previous studies (Li and Zhang, 2003; Liu et al., 2007) have shown that the viable cell numbers on GZ were commonly an order of magnitude higher than on PVC after 0.5

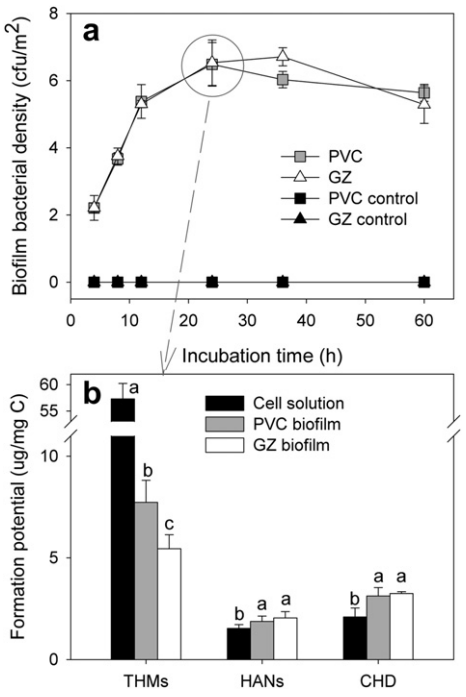

Fig. 3 – Biofilms formation of *Pseudomonas aeruginosa* on polyvinyl chloride (PVC) and galvanized zinc (GZ) surfaces changed with incubation time (a) and the disinfection byproduct formation potentials of 24-h biofilms compared to that of the pure *P. aeruginosa* cell solution (b). All error bars indicated the standard deviation of 3 replicates.

or 1 year incubation. Yet in this short-term study, the dependence of biofilm formation on different material surfaces did not show a large difference as previous reported.

3.2.2. *DBP formation*

The two types of pipe biofilms at maturation stage, and planktonic cell solution at stationary stage of *P. aeruginosa* (at 24 h incubation) were chlorinated based on UFC method to assess their DBP formation potentials, as marked in Fig. 3a. During the chlorination, the specific chlorine demand of PVC biofilm and GZ biofilm were 0.22 ± 0.18 and 0.19 ± 0.20 mg-Cl_2/mg-C, respectively, much lower than that of the planktonic cell solution (2.0 ± 0.20 mg-Cl_2/mg-C), also lower than any of seven other cell solutions from different bacterial species (on average 0.46–1.96 mg Cl_2/mg-C) in our previous study (Wang et al., 2012). Similarly, the specific THM formation potentials of PVC biofilm (7.7 ± 1.1 μg/mg-C) and GZ biofilm (5.4 ± 0.7 μg/mg-C) were lower than that of planktonic cell solution (57.3 ± 3.0 μg/mg-C) (*t*-test; $P < 0.01$), only amounting about 1/7 and 1/11, respectively (Fig. 3b). However, the HANs (PVC: 1.9 ± 0.3 μg/mg-C; GZ: 2.0 ± 0.3 μg/mg-C) and CHD (PVC: 3.1 ± 0.4 μg/mg-C; GZ: 3.2 ± 0.1 μg/mg-C) generated from these biofilms seemed to have larger production (*t*-test; $P < 0.01$) compared to those of the cell solution (1.5 ± 0.2 μg/mg-C for HANs and 2.1 ± 0.4 for CHD).

Here we first reveal that the phenotype (planktonic cells vs. biofilms) of the same bacterial species caused large difference in DBP formation. The formation/growth of biofilm is physiologically different from single-cells that float or swim in the liquid phase, and thus probably leads to the large variation of DBP precursor quality. Specifically, bacterial cells in the biofilm are commonly embedded within the extracellular polymeric substance (EPS) matrix, which contains high proportion of exopolysaccharides (Flemming and Wingender, 2001; Tsuneda et al., 2003; Wozniak et al., 2003; Flemming and Wingender, 2010). These carbohydrates have been shown to have much lower reactivity in THM and higher reactivity in CH formation (Hong et al., 2008; Navalon et al., 2008; Liu and Li, 2010; Wei et al., 2011), which should be the major reason causing different THMFPs from biofilm compared to planktonic cells. Besides, the surface EPS may also physically protect the cells within biofilm structure from reacting with disinfectants and enhanced their resistance to disinfectants (Lechevallier et al., 1988; Debeer et al., 1994), thereby resulting in lower specific chlorine demand and total DBPFP than planktonic cells.

3.3. *Effects of pipe materials on DBP formation from biofilms*

With increasing bromide addition in the reaction system containing bacterial materials (planktonic cell solution, biofilms of *P. aeruginosa*) and NaOCl, both the total THM and HAN mass were largely enhanced (Fig. 4a) and more brominated THM and HAN species were formed (Fig. 4b). Specifically, from 0 to 2.0 mg/L Br^- addition, the mean specific THM formation

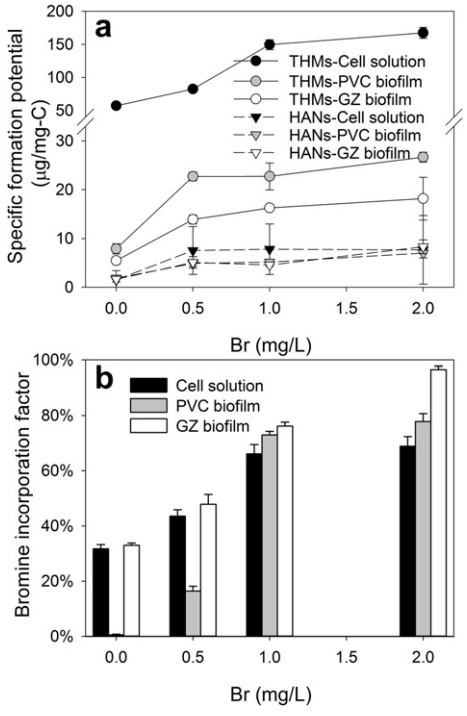

Fig. 4 – Trihalomethane and haloacetonitrile formation potentials of the *P. aeruginosa* biofilms in the presence of bromide (a), and the bromine incorporation fraction of trihalomethanes changed with increasing bromide concentration (b). All error bars indicated the standard deviation of 3 replicates.

potential increased 2.9, 3.4 and 3.3 times for planktonic cells, PVC biofilms and GZ biofilms, respectively, with the order of specific THMFP ranking unchanged: planktonic cells > PVC biofilms > GZ biofilms. As to mean specific HANFP, it increased 5.1, 3.1 and 4.9 times for planktonic cells, PVC biofilms and GZ biofilms, respectively. However, no significant difference (paired *t*-test; $P > 0.1$) were observed among HANFPs of planktonic cells, PVZ biofilms and GZ biofilms regarding the all four degrees of Br^- addition.

Brominated DBPs in drinking water have raised large concern due to its much higher carcinogenic risks compared to their corresponding pure chlorinated species (Richardson et al., 2003, 2007). Our results are consistent with many studies (Hua et al., 2006; Chow et al., 2007, 2008) showing that bromide is important DBP precursor that enhances the brominated fraction in DBP formation (Fig. 4). The formation of reactive bromine species from reactive chlorine and bromide should be the major reason for organic matter bromination and the brominated DBPs formation (Hua et al., 2006; Sivey et al., 2013). Notably, coastal water commonly contains massive bromide, and thus large fraction of brominated species in DBPs is expected to be formed when disinfecting bacteria in coastal water.

Although the biofilm growth did not differ in the pipe material (Fig. 3a), the DBP formation potentials from biofilm (Figs. 3b and 4a) and the BIF of THMs and HANs (Fig. 4b) appeared to depend on the pipe materials. Biofilms grew on the PVC consumed comparable chlorine, but produced more THM than those on GZ surface. This difference in THM formation was not likely to be attributed to the THMFP backgrounds of PVC and GZ materials (which have no significant difference), but may linked to the different biochemical composition in biofilms driven by pipe material effects. Notably, the PVC biofilms always presented lower bromine incorporation fraction of THMs compared to GZ biofilm (paired *t*-test; $P < 0.001$), with BIF difference ranging from 3 to 32%. Particularly, when no bromide was added, the BIF from PVC biofilm amount to only 0.5%, much lower than both those from GZ biofilm and planktonic cells (>30%). We speculated that this large difference may result from the altered Cl and Br equilibrium either by incorporation of Cl from PVC to biofilms or the reaction between reactive bromine and PVC which formed bromine-substituted PVC. As the first approach to evaluate the impact of pipe material on DBP formation from biofilms, this study has not yet addressed the mechanism(s) but it did suggest that the DBP speciation from biofilm was influenced by pipe materials. Besides this effect, the pipe materials can also influence the decay kinetic and availability of residual chlorine due to interactions with deposits, corrosion, and biomass at the inner pipes walls (Lechevallier et al., 1990; Lu et al., 1999), and thus may affect the yield of DBP formation. More future studies will be favorable to select and optimize the pipe material to reduce the health risks from biofilm itself and its derived DBPs.

3.4. *Environmental implications*

As bacteria in the source water are ubiquitous and they are revealed to be DBP precursors, their relative contribution to DBP formation in water disinfection has been overlooked. As drinking water sources, natural waters can have highly varied bacterial biomass, depending on the seasons and nutrient levels (i.e., eutrophic vs. oligotrophic) (Castillo et al., 2004). Although viable bacterial carbon accounts for <10% TOC in most natural water (Chrost et al., 2000), the natural waters in some regions could have rather high bacterial production, e.g., up to 85.5 $\mu g\text{-}C\ L^{-1}\ h^{-1}$ in Maumee River and 322 $\mu g\text{-}C\ L^{-1}\ h^{-1}$ in Ottawa River (Castillo et al., 2004). The fast transformation from non-microbial carbon to microbial carbon can provide massive alive and dead bacterial materials as DBP precursors. Whereas the bacterial contribution is highly varied when disinfecting natural water, it is consistently important in wastewater disinfection. In urbanized areas, wastewater is commonly biological treated and disinfected before discharge or reuse. Many studies (Namkung and Rittmann, 1986; Schulz and Hermann, 1998; Wei et al., 2011) have showed that the majority of the dissolved organic matter in effluents after biological treatment is actually soluble microbial products. Unintentional potable reuse of wastewater has often occurred after disinfection (Krasner et al., 2009), and therefore the bacterial contribution is crucial to the wastewater DBP formation and the accompanying health risks.

While it is extensively acknowledged that DBPs are formed *in situ* in the disinfection facilities, we showed the possibility of *ex situ* DBP formation in the water distribution systems, i.e., the biofilms reacting with residual chlorine. As biofilms are widely found in pipeline (up to 10^5–10^7 cfu/cm^2) and the water distribution systems have large contact area, contribution from biofilms to overall DBP budget in the finished drinking water cannot be ignored, especially when high residual chlorine exists. Although the biofilms showed lower specific DBPFP than planktonic cells, they can become source of planktonic cells after they reach to dispersion stage. It is notable that the release of planktonic cells into water from biofilms may therefore lead to an enhanced specific THMFP in the pipeline systems.

Our results suggested that bacterial materials can be important DBP precursors not only because their roles as previous unknown DBP sources but also their greater reactivity in more toxic N-DBP formation than other well-identified precursors such as humic substance and litter leachate (Wang et al., 2012). Both the planktonic cells and biofilms have high protein content (Tsuneda et al., 2003) and low organic C/N ratio, and consequently the bacteria-derived DBPs contained much larger fraction of HANs (here for *E. coli*, molar HANs/THMs ratio: 0.31–0.70; for *P. aeruginosa* biofilms, 0.19–0.37) than litter leachate (molar HANs/THMs ratio: 0.02–0.17 (Chow et al., 2011)), humic acids (molar HANs/THMs ratio: 0.01–0.04 (Reckhow et al., 1990)) or some wastewater effluents (molar HANs/THMs ratio < 0.1 (Tang et al., 2012)). Haloacetonitriles have been proved to be much more toxic compared to other common carbonous DBPs like THMs or HAAs (Muellner et al., 2007). In this case, compared to natural water rich in litter leachate or humic acids (high C/N ratio), disinfecting source water with massive bacterial cells (low C/N ratio) may result in a relatively higher HANs risk rather than THMs risk. Also, fast bacterial growth may promote carbon quality shift from non-microbial carbon to microbial carbon and result in high HANs formation in the subsequent disinfection process.

4. Conclusion

(1) We characterized DBP formation and bacterial survival of *E. coli* by different dosages of chlorination and chloramination. Most selected DBPs were detectable, including trihalomethanes, haloacetonitriles, chloral hydrate, chloropicrin, and 1,1,1-trichloro-2-propanone, with higher yield after chlorination compared to after chloramination. Also, the DBPs generated from bacterial materials had much larger haloacetonitrile fraction compared to other well identified precursors such as humic substance or litter leachate. A positive correlation ($P = 0.08–0.09$) between DBP formation and log reduction of bacteria implied that breaking down of bacterial cells released precursors for DBP formation.

(2) The effects of growth modes and the surface materials on DBP formation from *P. aeruginosa* were evaluated. Planktonic cells formed 7–11 times greater trihalomethanes per carbon of those from biofilms but significantly lower ($P < 0.05$) chloral hydrate, highlighting the bacterial phenotype's impact on the bacteria-derived DBPFP. Pipe material appeared to affect the DBPFP of bacteria, with 4–28% lower bromine incorporation factor for biofilms on polyvinyl chloride compared to that on galvanized zinc.

(3) Our study highlights the hitherto unknown roles of bacterial cells and biofilms as important DBP precursors in drinking water treatment systems. As prevalence of bacterial contamination in source waters and occurrences of biofilms in water treatment units and distribution systems are frequently reported worldwide, the contributions from bacterial sources to DBP risks cannot be ignored.

(4) More future studies on the biochemical characteristics of various bacterial materials, mechanisms and effects of environmental factors on bacteria-derived DBP formation by different disinfecting pathways, and association of bacterial carbon source and DBP occurrence in water treatment facilities, will further extend our knowledge on the bacterial contribution to health risks in drinking water.

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