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Formation and influencing factors of disinfection by-products from bacterial materials in drinking water distribution systems

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ABSTRACT

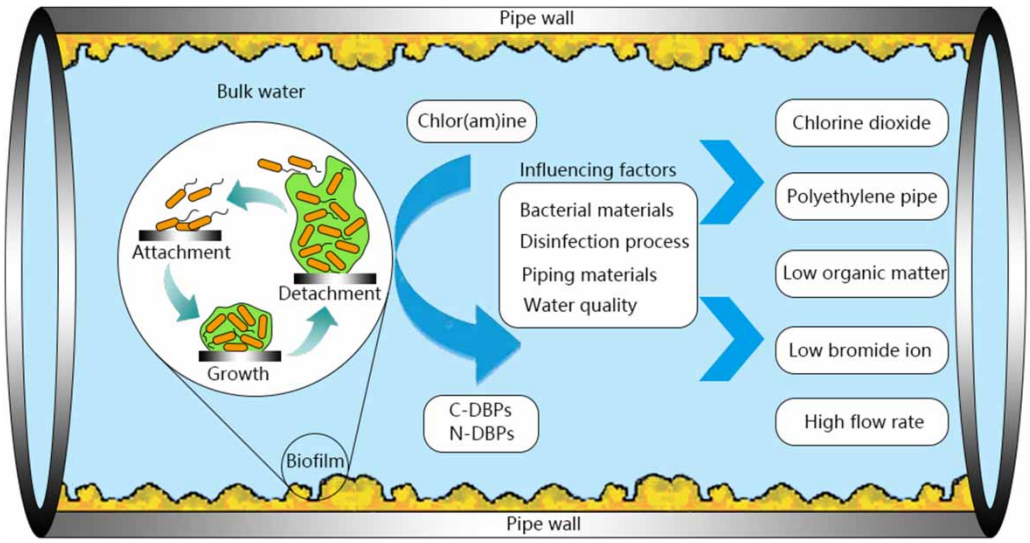
In recent years, the contribution of bacterial materials in water distribution systems to DBPs has attracted widespread attention from researchers. The risks and influencing factors of bacterial DBPs are reviewed. Factors affecting the generation of bacterial DBPs include the characteristics of bacterial materials, disinfection process, piping materials, and water quality. Among the major components of biofilms, proteins or amino acids have the greatest risk of DBPs. Among the commonly used disinfection methods, chlorine is more suitable for continuous disinfection of pipe networks than chloramine, but chlorine dioxide may be a better substitute in the future. Pipes with good biological stability, such as polyethylene (PE) pipe, should be encouraged to be used in water supply networks. Periodic removal of biofilms, reduction of organic matter and bromide ion concentrations, and maintenance of high flow rates are effective means to reduce bacterial DBPs. The control, removal and monitoring devices of tube wall biofilm need to be further developed and promoted. In the future, it is urgent to develop new disinfectants that are not easy to react with biomolecules and natural organic matter, and produce fewer or no by-products.

Key words: bacterial materials, disinfection by-products, drinking water distribution systems, influencing factors

HIGHLIGHTS

- Proteins or amino acids induce the greatest risk of DBP formation.
- Chlorine is more suitable for continuous disinfection of pipe networks than chloramine, but chlorine dioxide may be a better substitute.
- PE pipes should be encouraged to be used in water supply network.
- The concentration of organic matter and bromide ions in the water should be reduced as much as possible and the high flow rate of the water should be maintained.

GRAPHICAL ABSTRACT



ABBREVIATIONS

AOM	algal organic matter
BOM	bacterial organic matter
BDCM	bromodichloromethane
1,1-DCP	1,1-dichloro-2-propanone
1,1,1-TCP	1,1,1-trichloro-2-propanone
Cl-/Br-	chlorinated/brominated
CCPs	copper corrosion products
C-DBPs	carbonaceous disinfection by-products
CF	chloroform
CHD	chloral hydrate
CP	chloropropanones
CPK	chloropicrin
DBCM	dibronochloromethane
DBPs	disinfection by-products
DBPFP	disinfection byproduct formation potential
DCAA	dichloroacetic acid
DCAcAm	dichloroacetamide
DCAN	dichloroacetonitrile
DOM	dissolved organic matter
EPS	extracellular polymeric substances
FA	fulvic acid
HA	humic acid
HAAs	halogen acids
HAcAms	haloacetamides
HANs	haloacetonitriles
HPs	halonitromethanes
NAs	nitrosoamines
N-DBPs	nitrogenous disinfection by-products
NDMA	nitrosodimethylamine
NOM	natural organic matter
PVC	polyvinyl chloride
PE	polyethylene
TBM	tribromomethane
TCAcAm	trichloroacetamide
TCM	trichloromethane
TCNM	trichloronitromethane

THMs trihalomethanes
TOC total organic carbon
TOX total organic halogens
UTOX unknown total organic halogens

1. INTRODUCTION

Disinfection is a critical step in drinking water treatment and is extremely important for drinking water safety and public health. At present, most drinking water treatment plants use chlorine disinfection (including liquid chlorine and sodium hypochlorite disinfection), and a small number of water plants use chloramine disinfection to ensure the microbial safety of drinking water in the pipe network (Li *et al.* 2019). Since Rook (1974) first discovered disinfection by-products (DBPs) – chloroform (CF), produced during drinking water chlorination, more and more DBPs have been gradually discovered. With the gradual attention to the chemical safety of drinking water, more than 700 harmful DBPs have been detected in drinking water, most of which are carcinogenic, teratogenic, and mutagenic (Cai *et al.* 2018). Epidemiological investigations and laboratory studies have shown that halogenated DBPs such as trihalomethanes (THMs), haloacetic acids (HAAs), and haloacetonitriles (HANs) are cytotoxic and genotoxic, and long-term intake of DBPs-containing tap water increases cancer risk and have certain adverse effects on the human reproductive system (Shao *et al.* 2018). Plewa *et al.* (2007) used Chinese hamster ovary (CHO) cells to evaluate the cytotoxicity and genotoxicity of different DBPs, and found that the genotoxicity of DBPs ranked as follows: halonitromethanes (HPs: 7.4×10^3) > haloacetonitrile (HANs: 3.0×10^3) > haloacetic acids (HAAs: 5.0×10^2) > trihalomethanes (THMs: 1.5×10^2). The comprehensive toxicity (including cytotoxicity and genotoxicity) of nitrogenous disinfection by-products (N-DBPs) is much greater than that of carbonaceous disinfection by-products (C-DBPs). Since there are many types of DBPs, and many DBPs are currently unknown, only the most productive, hazardous and representative DBPs in the disinfection process could be selected for study. The studied C-DBPs mainly include THMs, HAAs, which represent the major classes of regulated DBPs. And N-DBPs mainly include HANs, haloacetamides (HAcAms), and HPs. Although the focus differs, all studies revolve around the main DBPs, because it is convenient and feasible to reflect the total DBP formation potential (DBPFP) in water by measuring the formation potential of the main DBP.

The proven DBP precursors include natural organic matter (NOM, such as humic acid (HA) and fulvic acid (FA)), algae, bacteria and other microorganisms. Traditional studies have listed NOM as the main precursor of DBPs, but recent studies have shown that disinfectants cannot only inactivate microorganisms in pipes, but also react with them to generate DBPs (Xu *et al.* 2018). Bacterial materials include planktonic bacteria distributed in bulk water and biofilms attached to pipe walls (Wang *et al.* 2013a). The study of DBPs and the control of bacteria and pipe wall biofilms have been regarded as two separate research fields for a long time. The contribution of pipe wall biofilms to DBPs is often overlooked (Krasner *et al.* 2006). In recent years, the effect of bacterial material on DBP formation has been widely reported (Wang & Hu 2018). The resulting DBPs are called 'disinfection by-products from bacterial disinfection' (Ng *et al.* 2015), and are called as bacterial DBPs briefly in this paper. Bacterial DBPs have been paid more and more attention by researchers, but there is little related literature.

This review summarizes the recent findings on bacterial DBPs. Literature searches were performed using databases such as Science Direct and Google Scholar. Keywords used for literature search included 'bacterial material', 'biofilm', 'EPS', 'disinfection by-products', 'influencing factors', 'drinking water distribution system', 'disinfection method', 'chlorine disinfection', 'chloramine disinfection', and 'pipe materials'. Studies related to the formation of DBPs from bacterial materials in water distribution systems were screened from the collected literature, and studies related to the removal of DBP precursors by biofiltration were excluded. The purpose of this review is to sort out the composition and DBP risk of bacterial materials and influencing factors under chlorine and chloramine disinfection methods in water distribution systems, and to propose effective measures to reduce bacterial DBPs and future research priorities in the field of drinking water safety.

2. COMPOSITION AND DBP RISK OF BACTERIAL MATERIALS

Bacteria mainly exist in the form of planktonic cells and pipe wall biofilms in the water supply network (Ruecker *et al.* 2007). The biomass in the water only accounts for 5% of the total biomass in the pipe network, and the biomass in the biofilm accounts for 95% (Flemming *et al.* 2002). Due to the great harm of pathogenic bacteria to the human body, the disinfection process is essential. Therefore, the formation of DBPs is inevitable in the process of inactivating bacterial cells by disinfectants (Delatolla *et al.* 2015). The disinfection process causes bacterial cells in the water to break down and release dissolved

organic matter (DOM) such as polysaccharides, proteins, and nucleic acids (Zhang *et al.* 2010a). Ng *et al.* (2015) refer to this as bacterial organic matter (BOM). Stevenson & Cole (1999) found that DOM from bacteria contains more nitrogen than NOM such as HA in water, because protein as an organic nitrogen source accounts for 50% to 60% of the dry weight of bacterial cells. Huang *et al.* (2013) found that the amount of N-DBPs generated by the chlorination of BOM (HANs: 28–37.2 nmol/mg TOC; HAcAm: 21–24.3 nmol/mg TOC) was higher than those generated by HA (HANs: 11.8 nmol/mg TOC; HAcAm: 10.6 nmol/mg TOC) with the same TOC. Therefore, although the concentration of bacteria in the bulk water is lower than that of HA, the risk of DBPs brought by bacteria cannot be ignored.

Biofilms are more resistant to disinfectants than planktonic cells, because extracellular polymeric substances (EPS) protect microorganisms from adverse environments and shear stress (Liu *et al.* 2016; Li *et al.* 2020). Therefore, in order to survive in the harsh environment of the pipe network, microorganisms usually need to adhere to the pipe wall of the water supply network in the form of biofilm. The surface biomass concentration of the pipe wall biofilm reach 10^4–10^7 CFU/cm^2 (Woolschlager *et al.* 2005). Unremoved NOM in the water distribution system can provide a nutrient source for the growth of pipe wall biofilms (Rodrigues *et al.* 2008). Pipe wall biofilms consist of aggregated microbial cells and EPS matrix (Flemming & Wingender 2010). The microorganisms that make up the biofilm are mainly bacteria, followed by archaea, fungi and protozoa (Fish *et al.* 2015), so this paper mainly discusses the bacteria and EPS in the biofilm. Almost all pathogenic bacteria in water can adhere to solid surfaces and form single-species biofilms under certain conditions (Wingender & Flemming 2011). Bacteria such as *Pseudomonas*, *Staphylococcus*, and *Methylobacterium* are known as biofilm-forming bacteria because they cannot only be incorporated into existing biofilms, but also actively attach to solid surfaces to form biofilms (Douterelo *et al.* 2014). So these bacteria are used by many researchers of bacterial DBPs to form biofilms. EPS secreted by bacteria *in vitro* contains nucleic acid, protein, polysaccharide, lipid and other biomolecules, accounting for more than 80% of the aqueous biofilm (Lemus Pérez & Rodríguez Susa 2017). At the same time, EPS is also an important repository for NOM such as humus (Wang *et al.* 2012b). EPS reduces the effectiveness of disinfectants and protects embedded bacteria, thereby enhancing the bio-instability of water distribution systems (Lee *et al.* 2015). Some bacteria are detached from the biofilm under the action of chlorine and shear stress, transformed into planktonic bacteria, and then attached to new surfaces or incorporated into other biofilms (Xue & Seo 2013).

Planktonic bacteria, biofilm-embedded bacteria, and EPS react with residual disinfectants in the water distribution system to generate DBPs. Biomolecules such as polysaccharides, proteins and nucleic acids contained in bacteria and EPS, as well as NOM (such as HA) adsorbed and stored in EPS, have been proved by many studies to form DBPs during the chlorination process (Li & Blatchley 2007; Hong *et al.* 2008; Navalon *et al.* 2008). The chlorine atom in hypochlorous acid produced by chlorine disinfection acts as an electrophile leading to electrophilic substitution of organic compounds. Proteins and amino acids form DBPs because they contain large amounts of phenolic or unsaturated/conjugated groups (Hu *et al.* 2020). Polysaccharides have also been shown to be important DBP precursors (Navalon *et al.* 2008). NOM such as HA contains various components such as resorcinol, carbohydrate and diketonic subunits. Most researchers believe that the chlorination of phenolic moieties in NOM is one of the reasons for the formation of THMs. The composition of bacterial material and its DBP risk are shown in Figure 1.

3. FACTORS INFLUENCING THE FORMATION OF BACTERIAL DBPS

3.1. The properties of the bacteria themselves

Different phenotypes of bacteria lead to different production of DBPs. Even the same bacteria are physiologically different when they exist in water in the form of biofilms and planktonic cells (Flemming & Wingender 2010). This can lead to differences in their chemical composition (Kives *et al.* 2006). Different biochemical compositions may result in different quality and quantity of DBPs (Li & Zhang 2003). Therefore, the contribution of the two bacterial phenotypes to DBPs needs to be discussed separately.

3.1.1. DBPs formed by planktonic bacteria

Bacteria present in the pipe network in the form of planktonic cells can react with residual disinfectants to generate bacterial DBPs. Several studies have shown that bacteria generate C-DBPs and N-DBPs during the chlorination process. Wang *et al.* (2012a) found that sodium hypochlorite solution reacted with some pure bacterial strains to generate CF, dichloroacetonitrile (DCAN), chloral hydrate (CHD), and their brominated analogs, suggesting that bacteria may be important precursors of DBPs in water. The yields of DCAN and CHD increased with the increase of bacterial concentration (calculated as TOC),

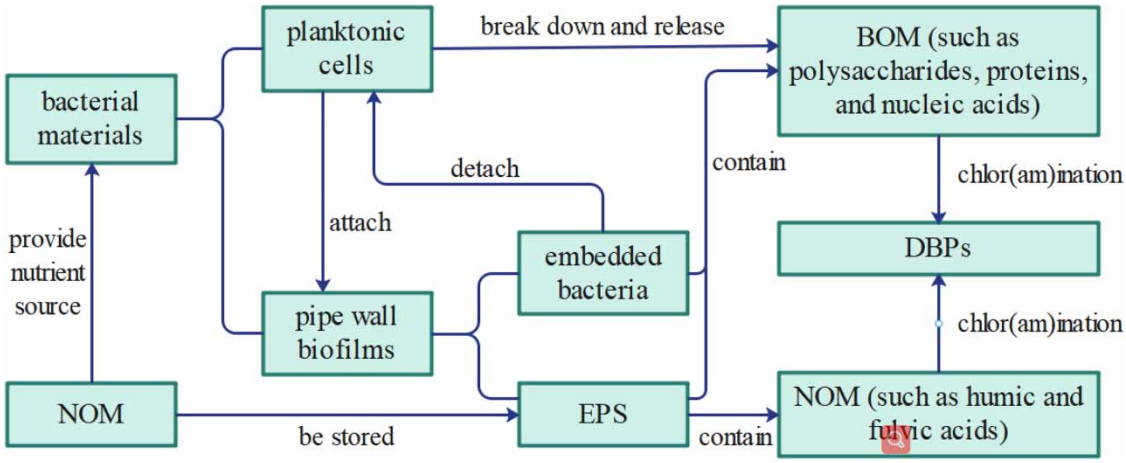


Figure 1 | The composition of bacterial material and its DBP risk.

while CF showed a fluctuating trend. This may be due to the different abilities of different biomolecules to form CF, and the changes in the relative abundance of biomolecules caused by bacterial metabolism significantly change the production of CF. Wang *et al.* (2013a) chlorinated and chloraminated *Escherichia coli (E. coli)* and detected THMs, HANs, CHD, chloropicrin (CPK) and 1,1,1-trichloro-2-propanone (1,1,1-TCP). The positive correlation between DBPs formation and *E. coli* log reduction suggests that decomposed bacteria release DBP precursors. Planktonic cells of *Pseudomonas aeruginosa* (*P. aeruginosa*) produced 7–11 times more THMs per carbon than biofilms, which may be due to the fact that the EPS wrapped on the outside of the bacteria contains a certain proportion of polysaccharides. The ability of polysaccharides to react with chlorine to form THMs is poor, and the ability to generate CHD is strong (Wang *et al.* 2013a).

Bacterial cells are one of the sources of organic nitrogen in water (Neidhardt *et al.* 1996). N-DBPs produced by planktonic bacteria are the focus of researchers. Huang *et al.* (2013) found that DCAN and dichloroacetamide (DCAcAm) generated from particulate matter in the secondary effluent of domestic sewage treatment plants accounted for 26–46% of the total production. Bacterial cells as part of the particulate matter are an important source of N-DBPs during chlorination in water treatment. *E. coli* Gram-negative bacteria, GNB) and *Enterococcus faecalis* (*E. faecalis*) (Gram-positive bacteria, GPB) suspensions produced more DCAN, DCAcAm and trichloroacetamide (TCAcAm) during chlorination than DOM in secondary effluent (Table 1). *E. coli* formed more DCAcAm and TCAcAm than *E. faecalis*, probably because GNB have thinner cell walls, which leads to more leakage of cellular material. However, *E. coli* formed less DCAN than *E. faecalis*, which may be related to differences in cell wall and cytoplasmic composition between GNB and GPB. Although specific differences were not discussed, Okayasu *et al.* (1997) found significant differences between the amino acid composition of *E. coli* and *Staphylococcus aureus* (*S. aureus*). *E. coli* contained more histidine ($1.96 \pm 0.15\%$) than *S. aureus* ($1.53 \pm 0.17\%$). Recent study has shown that histidine promotes the formation of HANs (Hu *et al.* 2020). Therefore, the difference in histidine content may be one of the reasons why GNB forms more DCAN than GPB. N-DBPs such as HANs and HAcAms have been shown to be more cytotoxic and genotoxic than traditional regulated DBPs (THMs, HAAs, etc.) (Plewa *et al.* 2008). The risk of N-DBPs brought by planktonic bacteria needs to be paid enough attention. The contribution of planktonic bacteria to DBPs is shown in Table 1.

3.1.2. DBPs formed by pipe wall biofilms

The transport and reactions of disinfectants and the formation of DBPs are affected by the inherent properties of biofilms. Rossman *et al.* (2001) found that the THMs content and chlorine consumption rate in the simulated pipeline environment were consistently higher than those in glass bottles, while the HAA content was not significantly different. This indicates that there are THMs precursor materials on the pipe wall, but no HAA precursor materials. But then a large number of studies have shown that the tube wall biofilm also has the potential to form HAAs (Table 2). The reason for no obvious change in HAAs content may be their degradation in water. Xu *et al.* (2018) respectively passed the effluent from three water treatment plants in Xiamen into a self-built PVC simulated household pipe loop for one year to form biofilms. The dismantled pipeline

Table 1 | The contribution of planktonic bacteria to DBPs

DBP precursors	Bacterial concentrations (calculated as TOC)	Disinfection method	Formation of DBPs	References
Pure bacterial strain	0; 0.083; 0.167; 0.333; 0.5 mmol·L^{-1}	Chlorine disinfection	$P_{DCAN、CHD} \propto$ Bacterial concentrations; P_{CF} shows a fluctuating trend	Wang *et al.* (2012a)
E. coli, *E. faecalis*	5 mg/L	Chlorine disinfection	$P_{N\text{-}DBPs}$(bacteria) > $P_{N\text{-}DBPs}$(DOM); P_{THMs}(bacteria) > P_{THMs}(DOM); P_{DCAN}(*E. faecalis*)(37.2 nmol/mg TOC) > P_{DCAN}(*E. coli*)(28.0 nmol/mg TOC) > P_{DCAN}(DOM)(11.8 nmol/mg TOC); P_{DCAcAm}(*E. coli*)(13.2 nmol/mg TOC) > P_{DCAcAm}(*E. faecalis*)(11.1 nmol/mg TOC) > P_{DCAcAm}(DOM)(4.3 nmol/mg TOC); P_{TCAcAm}(*E. coli*)(11.0 nmol/mg TOC) > P_{TCAcAm}(*E. faecalis*)(9.9 nmol/mg TOC) > P_{TCAcAm}(DOM)(6.3 nmol/mg TOC)	Huang *et al.* (2013)
E. coli	2 mg/L	Chlor(am)ine disinfection	THMs, HANs, CHD, CPK and TCP; *E. coli* log reduction$\propto P_{DBPs}$	Wang *et al.* (2013a)
Planktonic cells and biofilms of *P. aeruginosa*	1 mg/L	Chlor(am)ine disinfection	$P_{THMs/C}$(Planktonic cells) $\approx$ (7–11)$P_{THMs/C}$(biofilms)	Wang *et al.* (2013a)

Note: 'P' stands for DBPs production; '$\propto$' stands for positive correlation; '()' stands for DBP precursor; FP stands for DBP formation potential.

was exposed to an organic-free solution with a pH of 6.8 $\pm$ 0.2 and a free chlorine concentration of 4 mg/L for 48 h. The results showed that both the chlorine consumption and the THM production of the pipelines covered with biofilm were much higher than those of the pipelines without biofilm. Chlorine decay caused by biofilms in pipeline loops is more significant than those caused by water plant effluents. Therefore, the DBP risk of pipe wall biofilm needs to be paid enough attention.

Several studies have shown that both EPS and bacterial cells that make up biofilms can react with disinfectants to generate DBPs. Wang *et al.* (2012c) chlorinated *Pseudomonas putida* (*P. putida*) EPS (mainly protein) and *P. aeruginosa* EPS (mainly polysaccharide), and explored the effect of EPS composition and quantity on the production of DBPs. It was found that the reaction between EPS and chlorine would generate C-DBPs and N-DBPs. In the case of excess chlorine, the greater the amount of EPS, the greater the yield of DBPs (Wang *et al.* 2012c). Proteins and polysaccharides in EPS components have been shown to be important precursors of DBPs. Wang & Hu (2018) believed that polysaccharides are more likely to be the main precursors of DBPs, because after chlorine disinfection, the polysaccharide content in EPS was significantly reduced, and the protein content did not change significantly. However, this can only be taken as indirect evidence. The reduced proportion of polysaccharides in EPS does not directly indicate that polysaccharides are the main precursors of DBPs, as the conversion rate of polysaccharides to DBPs is probably lower than that of proteins. Wang *et al.* (2013b) extracted EPS from pure bacterial cultures (*P. aeruginosa* and *P. putida*) and mixed species biofilms isolated from water plants for chemical composition analysis, and determined the yield of chlorinated DBPs. The results showed that the DBP yield of amino acids was higher than that of polysaccharide monomers, and the DBP yield of polysaccharide monomers was less affected by its structure. This may be the reason why the proportion of polysaccharides is reduced more but protein is the main DBP precursor.

Although polysaccharides account for a larger proportion than proteins in biofilms, proteins, especially amino acids, have larger DBPFP (Table 2). This is because polysaccharides have a higher content of saturated carbon-ring structures than proteins, and the main precursors of DBPs are compounds with unsaturated/conjugated carbon bonds or phenolic structures. The protein in EPS contains more phenolic or unsaturated/conjugated groups, so it is more likely to form DBPs than the polysaccharides in EPS (Hu *et al.* 2020). Wang *et al.* (2012c) found that *P. putida* EPS produced twice as many N-DBPs as *P. aeruginosa* EPS because *P. putida* EPS contained more protein, i.e. more organic nitrogen, than *P. aeruginosa* EPS. This suggests that increased organic nitrogen content in EPS leads to increased production of N-DBPs (Wang *et al.*

Table 2 | The contribution of pipe wall biofilm to DBPs

DBP precursors	Maturation time of biofilm	Main component	Disinfection method	Formation of DBPs	References
Biofilms	1 year	Proteins, polysaccharides, lipids	Chlorine disinfection	P_{THMs}(Biofilms) > P_{THMs} (Bulk water)	Xu *et al.* (2018)
P. putida EPS	–	Proteins	Chlorine disinfection	$P_{HAAs} > P_{THMs}$; EPS production∝P_{DBPs}; $P_{N\text{-}DBPs}$(*P. putida* EPS) ≈ 2$P_{N\text{-}DBPs}$ (*P. aeruginosa* EPS)	Wang *et al.* (2012c)
P. aeruginosa EPS	–	Polysaccharides	Chlorine disinfection	$P_{HAAs} > P_{THMs}$; EPS production∝P_{DBPs}	Wang *et al.* (2012c)
EPS extracted from biofilms	–	Proteins, polysaccharides, lipids	Chlorine disinfection	P(Amino acids) > P(Polysaccharide monomers); $P_{HAAs} > P_{THMs}$	Wang *et al.* (2013b)
EPS extracted from biofilms	–	Proteins, polysaccharides	Chlor(am)ine disinfection	P(EPS) > P(Biofilm cells)	Wang & Hu (2018)
EPS extracted from biofilms	20 years	Proteins, polysaccharides, lipids	Chlorine disinfection	P(Proteins) > P(Polysaccharides); Amount of tyrosine∝P_{THMs}; Amount of histidine∝ $P_{HAAs, HANs, HAcAms}$; FP_{THMs}(Proteins) (198 μg/L) > FP_{THMs}(Polysaccharides) (50 μg/L); FP_{HAAs}(Proteins)(108 μg/L) > FP_{HAAs}(Polysaccharides) (27 μg/L); FP_{HANs}(Proteins)(31 μg/L) > FP_{HANs}(Polysaccharides) (1.5 μg/L); FP_{HAcAms}(Protein)(12 μg/L) > FP_{HAcAms}(Polysaccharides) (0 μg/L)	Hu *et al.* (2020)
Biofilms	1 year	Proteins, polysaccharides	Chlorine disinfection	P(Amino acids) > P(Polysaccharide monomers); FP(EPS) > FP(Biofilm cells)	Yan *et al.* (2022)
Biofilms	10 months	Proteins, polysaccharides, nucleic acids, lipids and NOM	Chlorine disinfection	Biofilm production∝P_{TOX}	Khan (2014)
Biofilms	168 days	Proteins (about 90%) and HA (about 10%)	Chlorine disinfection	Amount of tryptophan∝P_{DBPs}	Li *et al.* (2020)
Biofilms	1–2 years	Proteins, polysaccharides	Chlorine disinfection	Biofilm age∝FP_{DBPs}	Chen *et al.* (2020)

Note: 'P' stands for DBPs production; '∝' stands for positive correlation; '()' stands for DBP precursor; FP stands for DBP formation potential.

2012c). The types of amino acids have an important impact on the formation of DBPs. Histidine, alanine and tryptophan significantly contributed to the production of DBPs (Yan *et al.* 2022). In EPS protein, tyrosine had the strongest ability to promote the formation of THMs, and histidine had the strongest promotion effect on HAAs, HANs and HAcAms (Hu *et al.* 2020).

Studies have shown that the organic matter adsorbed and stored in the biofilm also has a certain influence on the composition of the biofilm and the formation of DBPs. Khan (2014) analyzed the generation of chlorinated DBPs and total organic halogens (TOX) from biofilms in a simulated water distribution system. The results showed that the formation of DBPs was significantly affected by the accumulation of organic matter (such as humus) in the biofilm matrix. The higher the density of the biofilm, the more TOX is formed. Li *et al.* (2020) used water containing humic substances (HS), algal organic matter (AOM) and agar (R2A) as feed solutions to culture biofilms in a reactor, respectively, and biofilm molecular properties and DBP formation was monitored for 168 days. The results showed that the protein content in all biofilm samples was

consistently about nine times the HS content, although the feed solutions contained different substances. Nonetheless, the composition of biofilms and the generation of DBPs still show dynamic changes over time, because the organic matter in the feed solution can be adsorbed by the biofilm and accumulate continuously. It gradually becomes part of EPS and is biodegraded. The adsorption and transformation processes of organic matter can affect the chemical composition of biofilms, thereby affecting the formation of DBPs. The contribution of amino acids and HS to DBPs formation differs in biofilms. There is a strong correlation between DBP formation and tryptophan-like substances, while HS with low molecular weight has a low tendency to form DBPs. In summary, among the major components of biofilms, proteins or amino acids have a greater risk of DBPs than polysaccharides and organic matter.

In addition, biofilm age is also thought to be associated with DBP risk. Chen *et al.* (2020) found that the number of bacteria on the wall of pipe that had been used for two years was significantly higher than those on the wall of pipe that had been used for one year, and DBPFP of the two-year-old pipe was 50% higher than that of the one-year-old pipe. The new pipe can form biofilms and pose certain biological and chemical risks within a short period of time when it is put into use. It is very necessary to study the method of delaying the pollution of new pipelines and improve the effect of pipeline network renewal. The contribution of pipe wall biofilm to DBPs is shown in Table 2.

Overall, bacterial species, number, phenotype, EPS composition and number, and biofilm age all affected DBP production. The bacterial DBPs mainly include C-DBPs (THMs, HAAs, CHD, halogenated ketones (HKs), etc.), N-DBPs (HANs, HAcAm, HPs, CPK, etc.). These DBPs pose a significant risk to the safety of drinking water. The effect of bacterial species on EPS composition indicated that the effect of disinfection methods on bacterial community structure in water was not negligible. In the disinfection process, on the basis of ensuring the biological safety of drinking water, the proportion of bacteria that produce EPS containing more protein or amino acids should be minimized. In practice, however, direct control of EPS composition is very difficult. In the actual network, environmental factors such as the type and dose of disinfectant, pipe material and water quality can lead to changes in the dominant genus of bacteria in the network, which can result in changes in EPS composition. Therefore, the composition of EPS can be altered by controlling environmental factors that affect bacterial growth. The protein content of EPS in the pipe network may be an indicator for assessing the risk of DBPs in the pipe network. An increase in bacterial concentration and biofilm formation time leads to an increase in DBPs, but does not change the DBP production pattern. The bacterial concentration, EPS and biofilm all contribute to the formation of DBPs, suggesting that regular removal of biofilm from the pipe wall and the development of more efficient methods for monitoring and removing biofilms are necessary.

3.2. Disinfection process

3.2.1. Disinfection methods

Disinfection methods have a significant effect on the formation of bacterial DBPs. At present, the disinfection method to maintain the biological stability of the pipeline network is mainly chlorine disinfection, followed by chloramine disinfection. Wang & Hu (2018) found that bacterial materials produced more DBPs than NOM during chlorination, but less DBPs than NOM during chloramination. Wang *et al.* (2020) also found that the level of DBPs formed by chloramine was much lower than those formed by chlorine and was not significantly affected by the residual amount of chloramine. But chlorine is more powerful than chloramine in removing biofilms. When the free chlorine concentration was the same, the average thickness and total biomass of the biofilm formed in the reactor during chlorine disinfection ($3.83 \pm 2.38\ \mu m$; $5.87 \pm 2.35\ \mu m^3/\mu m^2$) were less than those during chloramine disinfection ($7.82 \pm 3.18\ \mu m$; $7.72 \pm 1.50\ \mu m^3/\mu m^2$). Duan *et al.* (2020) compared the C-DBPs (THMs and HAAs) and N-DBPs (nitrosamines, NAs) yield and toxicity. The results showed that pre-ozonation increased bacterial DBPFP and calculated toxicity. This is because the high-molecular-weight fraction in water is converted to a low-molecular-weight fraction that reacts easily with the disinfectant during the pre-ozone process. At the same time, the bacteria release more intracellular organic matter (EOM) to react with the disinfectant in the presence of ozone. The high level of nitrosodimethylamine (NDMA) produced by the ozone process is an important reason for the increased calculated toxicity of DBPs. Although chlorine and ozone/chlorine have higher DBPFPs, the resulting DBPs are calculated to be less toxic than chloramine-based disinfection methods. This is because chloramine generates more N-DBPs, which are more cytotoxic and genotoxic than C-DBPs.

Chlorine disinfection is more suitable than chloramine disinfection for continuous disinfection of pipeline networks, both in terms of inhibiting biofilm growth (Wang *et al.* 2020) and reducing the toxicity of DBPs (Duan *et al.* 2020). When ozone is used for pre-oxidation, the residual ozone dose should be carefully monitored and controlled in order to reduce the toxicity of

DBPs. Although chlorine disinfection is better for the biochemical safety of distribution networks, it is still not the best choice. Chlorine dioxide has been shown to be more effective than chlorine or chloramines against planktonic bacteria and biofilms (Chauret *et al.* 2005), with lower production of halogenated DBPs (Serrano *et al.* 2015), and lower toxicity (Han & Zhang 2018). However, there is no evidence that chlorine dioxide is associated with bacterial DBPs and therefore further research is required on the interaction of chlorine dioxide with bacterial materials.

3.2.2. Disinfectant dose and contact time

Numerous studies have shown that the dose and contact time of the disinfectant also have a significant effect on the formation of DBPs. Ng *et al.* (2015) found that 0.5 mg/L of chlorine produced less THMs than 1.5 mg/L of chlorine after chlorinating *E. coli* solutions for 80 min. Increased chlorine dose increases disinfection efficiency, but also increases the risk of DBPs. Huang *et al.* (2013) used 10 mg/L chlorine to disinfect the bacterial suspension and found that the DCAN concentration increased continuously before 72 h and decreased after 72 h. This may be because DCAN can be hydrolyzed to DCAcAm. The yields of DCAcAm and TCAcAm increased with contact time. This may be because the DOC leached from bacterial cells increases with chlorination time, resulting in an increase in DBP precursors. When different concentrations (0–50 mg/L) of chlorine were used to disinfect the bacterial suspension for 24 h, it was found that the concentrations of DCAN and DCAcAm first increased and then decreased with chlorine dose increasing, while the concentration of TCAcAm increased continuously with chlorine dose increasing. This may be because the hydrolysis rates of DCAN, DCAcAm and TCAcAm all increased with the increase of chlorine dosage, and a higher chlorine dosage was required to increase the hydrolysis rate of TCAcAm. Then Li *et al.* (2016) found in the process of chlorination of *E. coli* that trichloromethane (TCM), 1,1-dichloro-2-propanone (1,1-DCP), trichloroacetonitrile (TCAN), TCNM, DCAA, and TCAA all increased with the increase of chlorine concentration and contact time.

The relationship between the composition of EPS and the formation of DBPs varies at different chlorine doses. Lemus-Pérez & Rodríguez Susa (2017) exposed biofilms to high-dose (2.6 ± 0.8 mgCl/L) and low-dose (0.7 ± 0.2 mg Cl/L) chlorine disinfectants, and performed a correlation analysis between EPS components and DBPs. Higher levels of aromatic proteins (tryptophan, tyrosine, valine and arginine-like substances) and less humic and soluble proteins were detected in both cases. EPS contains more aromatic proteins at high chlorine concentrations than those at low chlorine concentrations. Br-HAA, dibromoacetonitrile (DBAN), bromochloro acetonitrile (BCAN) are negatively correlated with arginine and valine. This is probably because aromatic proteins do not react with chlorine to form DBPs. Proteins in EPS may not be related to DBPFP, while DNA, rhamnolipids or polysaccharides may be the main precursors. At low chlorine concentrations, the biofilm adsorbed more organic matter due to the low bactericidal effect of chlorine. Amino acids (mainly alanine, threonine, proline and isoleucine) and HS content in EPS were relatively higher. Alanine was positively correlated with Br-HAA, CF, N-DBPs (CPK, DCAN, TCAN) and valine and HS were positively correlated with CF. In a subsequent experiment, Lemus-Pérez & Rodríguez Susa (2020) found that high chlorine doses resulted in high DBPFP (Table 3). The order of DBPs concentrations was: HAAs > THMs > CP (chloroacetone) > HANs > CPK. High concentrations of chlorine (2.6 ± 0.8 mg Cl/L) promoted Cl-HAA and reduced Br-HAA, whereas the opposite was true for low concentrations (0.7 ± 0.2 mg Cl/L). Because a high Cl/DOC ratio promotes the formation of Cl-HAA and reduces the bromine incorporation factors (BIF) of HAAs. Duan *et al.* (2020) found that the amount of THMs and HAAs formed by bacterial materials increased with disinfectant dose increasing, while the production of NDMA was not significantly correlated with the dosage of disinfectant. These studies have shown that increasing the chlorine dose promotes the formation of most DBPs. However, reducing chlorine dosage increases the microbial risk of the pipe network. A balance should be sought between biofilm control and minimization of DBPs to optimize the disinfection solution.

Wang *et al.* (2020) used a biofilm bioreactor to simulate a water distribution system and investigated the formation and decay of DBPs from biofilms under chlor(am)ine disinfection. The water in the reactor was disinfected with 0.5, 2, and 4 mg/L of chlor(am)ine for 50 d (the first stage), 25 d (the second stage), and 25 d (the third stage), respectively. The results showed that 0.5 mg/L of chlorine could not effectively remove the biofilm. 2 mg/L of chlorine caused a sharp rise in DBPs within 15 days due to increased reaction with biofilms. THMs and HAAs are dominant DBPs. However, with the removal of biofilm, the formation of DBPs gradually decreased and stabilized. DBPs did not increase further even when the chlorine concentration reached 4 mg/L. This result differs from that shown by the simulated pipeline system (Lemus-Pérez & Rodríguez Susa 2017). It could be because the biofilm reactor operated for a longer period of time than the simulated pipeline system. It could also be because the biofilm reactor does not fully reflect the actual conditions of the water supply network.

Table 3 | The effect of disinfection process on the formation of DBPs

Disinfection method	Disinfectant dose (mg Cl/L)	Type of device	Contact time	DBP precursors	Formation of DBPs	References
Chlor(am)ine disinfection	10	A new batch biofilm reactor consisting of only glass material	7 d	Biofilms	Chlorination: P(bacterial materials) > P(NOM); Chloramination: P(NOM) > P(bacterial materials)	Wang & Hu (2018)
Chlor(am)ine disinfection	0.5, 2,4	Continuously-fed CDC biofilm reactors (Boltzmann, MT, USA)	Operating time: 50, 25, 25 d	Biofilms	$P(Cl_2) > P(NH_2Cl)$; 0.5 mg Cl/L: The biofilm could not be removed effectively; 2 mg Cl/L: DBPs rose sharply within 15 days, and gradually decreased and stabilized; 4 mg Cl/L: DBPs did not increase further	Wang *et al.* (2020)
Chlor(am)ine, ozone/chlor(am)ine disinfection	O_3: 1 (mg O_3/L); Cl_2, NH_2Cl: 0, 5.0, 10.0, 20.0	Glass bottles	7 d	Biofilms	$P_{THMs,\ HAAs} \propto$ Disinfectant dose; $FP(Cl_2) > FP(NH_2Cl)$; Calculated toxicity: $O_3 > > O_3/NH_2Cl > NH_2Cl > O_3/Cl_2 \approx Cl_2$	Duan *et al.* (2020)
Chlorine disinfection	0.5, 1.5	Glass-topped amber bottle	80 min	*E. coli*	0.5 mg Cl/L: $P_{THMs} = 0.8$–5.3 μg/L; 1.5 mg Cl/L: $P_{THMs} = 1.1$–40.1 μg/L	Ng *et al.* (2015)
Chlorine disinfection	10	Amber glass bottles	0.5–144 h	*E. coli*, *E. faecalis*	DCAN: Increase first then decrease; DCAcAm, TCAcAm: Increasing	Huang *et al.* (2013)
Chlorine disinfection	0–50	Amber glass bottles	24 h	*E. coli*, *E. faecalis*	DCAN, DCAcAm: Increase first then decrease; TCAcAm: Increasing	Huang *et al.* (2013)
Chlorine disinfection	2–20	Reaction bulb	6–72 h	*E. coli*	TCM, 1,1-DCP, TCAN, TCNM, DCAA, TCAA: Increasing with chlorine concentration and contact time DCAN: Increase first and then decrease with chlorine concentration and contact time increasing	Li *et al.* (2016)
Chlorine disinfection	2.6 ± 0.8, 0.7 ± 0.2	A simulated piping system	48 h	EPS extracted from biofilms	2.6 ± 0.8 mg Cl/L: $FP_{THMs} = 15.94$ μg/mg C; $FP_{HAAs} = 35.18$ μg/mg C; 0.7 ± 0.2 mg Cl/L: $FP_{THMs} = 10.17$ μg/mg C; $FP_{HAAs} = 28.07$ μg/mg C	Lemus-Pérez & Rodríguez Susa (2017)
Chlorine disinfection	2.6 ± 0.8, 0.7 ± 0.2	A simulated piping system	48 h	EPS extracted from biofilms	2.6 ± 0.8 mg Cl/L: $FP_{THMs} = 0.75$–99.15 μg/mg C; $FP_{HAAs} = 7.45$–70.54 μg/mg C; 0.7 ± 0.2 mg Cl/L: $FP_{THMs} = 5.16$–122.3 μg/mg C; $FP_{HAAs} = 1.7$–48.98 μg/mg C	Lemus-Pérez & Rodríguez Susa (2020)

Note: 'P' stands for DBPs production; '∝' stands for positive correlation; '()' stands for DBP precursor; FP stands for DBP formation potential.

2–4 mg/L of chloride is seldom used in public drinking water supply systems and not practical either. It can work in a bioreactor but not in a real-life system. Furthermore, high doses of chlorine can lead to the regeneration of chlorine-resistant, pathogenic or conditionally pathogenic bacteria in the network and increase the microbiological risk of the network (Shekhawat *et al.* 2020). Therefore, increasing the chlorine dose is not an effective means of reducing the risk of biofilms and bacterial DBPs. In order to protect the safety of drinking water systems, the regulatory agency has set a limit value of 0.5 mg/L for free chlorine concentration and 30 min for contact time (WHO 2011). This limit value is still a reasonable dose at present.

Although chlorine disinfection has a larger DBPFP than chloramine disinfection, chloramine has a greater toxicological risk than chlorine due to the larger N-DBP production. At the same time, the strong biofilm removal ability of chlorine makes chlorine-based disinfection methods more suitable for continuous disinfection of pipeline networks. Pre-ozonation had a certain promoting effect on production of bacterial DBPs during chlor(am)ine disinfection. Nonetheless, the use of pre-ozonation should still be comprehensively assessed in combination with other factors in the water treatment process. Chlorine dioxide has a high biofilm removal capacity and low toxicity. If cost and storage issues can be addressed, chlorine dioxide may become a better alternative to chlorine and chloramines in the future.

3.3. Pipe material

There are significant differences in the growth of pipe wall biofilms due to differences in pipe materials (Zhang *et al.* 2017). Jang *et al.* (2011) explored the biofilm formation of steel pipes, copper pipes, polyvinyl chloride (PVC) pipes and stainless steel pipes within 4 months of operation, and found that at the end of the operation, the bacterial concentration of biofilm on the steel pipe was the highest (10^5 CFU/cm^2), and is about 100 times higher than other pipes. Due to the promoting effect of bacteria on copper corrosion, the bacterial concentration of biofilm on the copper pipe (8.87×10^3 CFU/cm^2) was higher than that on the stainless steel pipe (3.25×10^3 CFU/cm^2) at the end of the run. Since PVC dissolved compounds contain organic materials that are conducive to bacterial growth, the bacterial concentration of biofilm on PVC pipe (4.0×10^3 CFU/cm^2) is between that of copper pipes and stainless steel pipes.

The differences in biofilm formation caused by different pipe materials can lead to differences in the formation of DBPs. Chen *et al.* (2020) built a pilot platform at the front and end of the water distribution system and conducted a two-year experiment on four different pipes in order to explore the growth status of pipe wall biofilms and DBPFP. It was found that steel pipes had the highest DBPFP, followed by ductile iron pipes, galvanized steel pipes and stainless steel pipes. This indicates that ordinary steel pipes contribute to the rapid formation of bacterial DBPs, while stainless steel pipes have the best effect on maintaining the biochemical stability of the pipe network. Wang *et al.* (2013a) found that biofilms on PVC pipes generated more THMs than those on galvanized steel pipes, while TCAN and CHD were less generated than galvanized steel pipes. This may be because the PVC pipe wall is relatively smooth, which is not conducive to the adhesion of biofilms. Biofilms are easily shed by external forces such as hydraulic scouring, thus losing the protection of EPS and turning into planktonic bacteria. This process increases the production of THMs. The surface of galvanized steel pipe is rough, and bacteria are more likely to adhere to the pipe wall and secrete EPS. This resulted in more TCAN and CHD production from biofilms on galvanized steel pipes. Compared with PVC pipes, galvanized steel pipes have stronger ability to generate Br-DBPs and N-DBPs and are more harmful. Therefore, its use should be minimized. Khan (2014) analyzed the biofilm formation of iron and polycarbonate pipes in a simulated water distribution system and found that the biofilm activity of iron pipes was about 1.5 times that of polycarbonate pipes. Corrosion of the iron pipes increases the biofilm growth opportunities and adsorption sites for HS, leading to an increase in DBPs and unknown total organic halogens (UTOX). Yan *et al.* (2022) used a biofilm reactor with pipe coupons to explore the contribution of biofilms formed by different pipe materials (ductile iron pipes, cement-lined stainless steel pipes, polyethylene (PE) pipes) to DBPs. Both C-DBPFP and N-DBPFP were found to be the highest in the biofilm on the ductile iron pipe wall. This indicates that ordinary iron pipes and ductile iron pipes can aggravate the increase of bacterial DBPs, which will bring potential threats to human health.

Copper pipes are widely used in water distribution systems due to the generation of Cu^{2+} which has a certain passivation effect on certain bacteria. But research has shown that metal oxides shed into the water from the inner surfaces of pipes can have detrimental effects on water quality (Price & Jefferson 1997). Hu *et al.* (2020) investigated the catalytic effect of copper corrosion products (CCPs, including CuO and Cu^{2+}) on C-DBPs and N-DBPs generated from EPS chlorination. The results showed that CCPs significantly promoted the formation of DBPs, especially N-DBPs. The homogeneous catalysis induced by the complexation of Cu^{2+} with EPS and the heterogeneous catalysis induced by the polarization of Cl atoms in CuO and $HOCl/OCl^-$ were the main reasons for the increase of DBP. This indicates that although copper tube has a certain antibacterial effect, the promoting effect of its oxidation products on DBPs cannot be ignored.

The pipe material has an important influence on the formation of biofilms and DBPs. Pipes such as steel pipes, iron pipes, and ductile iron pipes have a greater contribution to biofilms and bacterial DBPs. Due to the advantages of low cost, high strength, corrosion resistance, and convenient construction, ductile iron pipes are widely used in various regions of China, but they have a greater risk of bacterial DBPs. Copper pipes have been widely used in Europe and the United States as

water supply pipes, and are also considered to have certain biological stability. However, its corrosion products can promote the formation of DBPs, which cannot be ignored.

From the perspective of avoiding the formation of bacterial DBPs, stainless steel pipes, PVC pipes and PE pipes are more suitable. However, the use of stainless steel pipes is limited by its own cost. Compared with traditional pipes, PVC and PE pipes have the advantages of light weight, corrosion resistance, low resistance, energy saving, rapid installation, long service life and low cost, and have been widely promoted and applied in urban water supply projects. According to the available data, the DBPFP of THMs and HANs in PVC pipes (7.7 mg/mg C; 1.9 mg/mg C) was slightly lower than that in PE pipes (9.0 mg/mg C; 2.1 mg/mg C), but the difference was small (Table 4). This indicates that the ability of the two pipes to reduce DBPs differs very little. Due to the rapid development of PE pipe, it gradually got rid of the disadvantage of poor economy and showed a trend of surpassing PVC pipe. PVC is also more liable to leach harmful substances into the water, either vinyl-chloride monomers or lead used as a plasticizer. The development prospects of PE pipes will be very broad, and they have great advantages in ensuring the biochemical safety of drinking water, and are worthy of being encouraged to use.

3.4. Water quality

3.4.1. Water velocity and temperature

Study has shown that the turbulent state at low residual chlorine concentration leads to an increase in the number of bacteria in the biofilm, possibly because the high flow rate increases the mass transfer of nutrients in the biofilm (Lehtola *et al.* 2006). The water flow can produce a certain shear stress on the biofilm. Low shear stress can remove the outer biofilm and some sediments in the distribution network, but even higher shear stress has difficulty removing the more cohesive, denser biofilm (Liu *et al.* 2017). With the increase of chlorine concentration, the high shear stress caused by high flow rate promotes the separation of biofilms and enhances the disinfection effect by promoting the permeation of chlorine in the biofilms (Tsai 2006). Abokifa *et al.* (2016) established a model and found that flow rate can significantly affect the formation of THMs from biofilms. Increasing the flow rate can accelerate the mass transfer of the disinfectant and matrix at the biofilm interface. When the Reynolds number (Re) was in the range of 5,000–15,000, the production of THMs increased with the increase of the flow rate; when the Re was in the range of 25,000–30,000, the production of THMs decreased with the increase of the flow rate. Flow rate in water distribution system should minimize biofilm and DBP formation. In addition to regular flushing and cleaning of the pipe network, it is also necessary to maintain a high flow rate in the water distribution system within a reasonable range.

Abokifa *et al.* (2016) also found that increased water temperature caused multiple reactions to proceed simultaneously. This process can promote the reaction of chlorine and organic matter, and accelerate the decay of residual chlorine. On the one

Table 4 | The effect of pipe materials on the formation of DBPs

Number	Formation of DBPs	References
1	Bacterial concentration in biofilm: steel pipe > copper pipe > PVC pipe > stainless steel pipe	Jang *et al.* (2011)
2	DBPFP: steel pipe (82.5–174.6 μg/L) > ductile iron pipe (87.3–159.9 μg/L) > galvanized steel pipe (73.51–152.9 μg/L) > stainless steel pipe (57.0–94.6 μg/L)	Chen *et al.* (2020)
3	FP_{THMs}: PVC pipe (7.7 ± 1.1 mg/mg C) > galvanized steel pipe (5.4 ± 0.7 mg/mg C); FP_{TCAN}: galvanized steel pipe (2.0 ± 0.3 mg/mg C) > PVC pipe (1.9 ± 0.3 mg/mg C); FP_{CHD}: galvanized steel pipe (3.2 ± 0.1 mg/mg C) > PVC pipe (3.1 ± 0.4 mg/mg C);	Wang *et al.* (2013a)
4	$P_{DBPs,\ UTOX}$: iron pipe > polycarbonate pipe; Biofilm activity: iron pipe ≈ 1.5 polycarbonate pipe	Khan (2014)
5	Biofilm biomass: ductile iron pipe > cement-lined stainless steel pipe > PE pipe; C-DBPFP: ductile iron pipe > cement-lined stainless steel pipe > PE pipe; N-DBPFP: ductile iron pipe > PE pipe > cement-lined stainless steel pipe; FP_{THMs}: ductile iron pipe (14.7 mg/mg C) > cement-lined stainless steel pipe (13.0 mg/mg C) > PE pipe (9.0 mg/mg C); FP_{HAAs}: ductile iron pipe (8.8 mg/mg C) > cement-lined stainless (7.9 mg/mg C) > PE pipe (5.4 mg/mg C); FP_{DCAN}: ductile iron pipe (2.4 mg/mg C) > PE pipe (2.1 mg/mg C) > cement-lined stainless (1.9 mg/mg C); FP_{DCAcAm}: ductile iron pipe(1.9 mg/mg C) > PE pipe (1.6 mg/mg C) > cement-lined stainless (1.4 mg/mg C); FP_{TCNM}: cement-lined stainless (2.1 mg/mg C) > ductile iron pipe (2.0 mg/mg C) > PE pipe (1.6 mg/mg C);	Yan *et al.* (2022)
6	CCPs can significantly promote the formation of DBPs, especially N-DBPs	Hu *et al.* (2020)

Note: 'P' stands for DBPs production; FP stands for DBP formation potential.

hand, the rising water temperature accelerates the utilization rate of the substrate by bacteria, thereby promoting the growth of bacteria. On the other hand, increases in temperature accelerate the reaction rate of chlorine with bacteria, thereby accelerating bacterial death and THM formation. The production of THMs increased continuously between 0 and 25 °C. Even though the number of bacteria at 20 °C was greater than that at 25 °C, the production of THMs was still the greatest at 25 °C. This indicated that elevated water temperature promoted the formation of bacterial DBPs (Abokifa *et al.* 2016). Zeng *et al.* (2021) came to the opposite conclusion when exploring the relationship between bacteria, DOC and DBPFP in natural water. Regardless of bacterial concentration, DOC, THMFP and HAAFP showed a decreasing trend with increasing temperature (15, 25, 35 °C). This may be because higher temperature increases bacterial activity, leading to an increase in DOC consumption and a decrease in DBPFP. However, this view only considers the effect of temperature on DBPFP when bacteria and DOC coexist, and does not consider the effect of temperature on the reaction and decay rate of chlorine when chlorine coexists. Because the DBPFP standard method 5710 was used in the experiment to measure the DBPFP of water samples containing microorganisms and DOC, and the measurement process was not affected by temperature. In the actual disinfection process, the increase in temperature increases the reaction rate and decay rate of chlorine, resulting in an increase in DBPFP. Since temperature changes are more affected by seasons, it is difficult to artificially control the temperature of the water distribution system. Therefore, more attention should be paid to the reasonable regulation of flow rate.

3.4.2. pH and impurities in water

The chemical properties of water (pH, organic matter, bromine, calcium and magnesium ion concentrations) have an effect on the formation of bacterial DBPs. There are some conflicting views on the effect of pH on bacterial DBPs. Huang *et al.* (2013) found that DCAN, DCAcAm, and TCAcAm were more easily formed at neutral pH during the chlorination of bacterial suspensions, while exhibited lower concentrations at pH 5 and 9. This may be due to the poor reactivity of chlorine with HANs and HAcAms precursors under acidic conditions, and the maximum hydrolysis rates of HANs and HAcAms under alkaline conditions. Ng *et al.* (2015) also found that a lower pH during the chlorination of bacterial suspensions could help improve disinfection efficiency and reduce the generation of DBPs. Therefore, the chlorination process should be controlled under low pH conditions. Although Li *et al.* (2016) also believed that the reduction of DBPs under alkaline conditions was due to hydrolysis, it is believed that the pH of chlorination should be controlled to be 9, because the production of N-DBPs is the least when the pH is 9. It has also been suggested that an increase in pH promotes the formation of DBP. Hu *et al.* (2020) found that an increase in pH would promote the electrostatic interaction of CCPs with EPS and HOCl/OCl^- to significantly increase DBPs. In conclusion, the optimal pH control conditions are still controversial. A balance should be sought between ensuring disinfection efficiency and reducing the risk of DBP to obtain optimal pH conditions. Further research on the effect of pH is needed in the future.

Zhang *et al.* (2010b) found that an increase in Br^- concentration would increase the brominated components in THMs and HANs produced by bacterial chlorination, and decrease the chlorinated components. This may be because Br^- is converted to HOBr during the chlorination process of bacteria. HOBr is more oxidative and has a longer retention time than HOCl. The bromination reaction was superior to the chlorination reaction resulting in the increase of Br-DBPs. Wang *et al.* (2013a) reached the same conclusion in a subsequent study. Br-DBP has been highly valued by researchers because of its greater carcinogenic risk than Cl-DBPs. Therefore, the water treatment process should minimize the Br^- concentration of the effluent.

Wang & Hu (2018) studied the DBPFP of biofilm cells, EPS matrix and NOM in desalinated seawater transport pipeline network. The results show that remineralization of desalinated seawater (addition of calcium and magnesium ions) can increase bacterial DBPFP, especially when magnesium ions are added. This may be because calcium and magnesium ions convert low molecular weight components of bacterial material to more hydrophobic, larger molecular weight components. Zhao *et al.* (2016) also believed that chlorination of high hardness water would generate more DBPs. When NOM (HA and FA) were used as DBP precursors, 4 mM calcium ions increased the production of TCM, DCAA, and TCAA by 47, 61, and 25%, respectively. This may be related to the complexation between Ca^{2+} and NOM. When low molecular weight NOM substitutes (including pyruvate, citric acid and other small molecular compounds) were used as precursors, 4 mM calcium ions increased the production of DCAA, TCAA and TCM to varying degrees. This indicates that the molecular structure and molecular weight of DBP precursors do not affect the catalytic effect of Ca^{2+}. However, Ta *et al.* (2020) came to the opposite conclusion using HA as a DBP precursor. When the Ca^{2+} concentration increased from 0 to 900 mg/L, the concentrations of THMs and tribromomethanes (TBM) decreased from 138 and 136 to 47.1 and 36.0 μg/L, respectively. The concentrations of the other three THMs (TCM, bromodichloromethane (BDCM) and dibronochloromethane (DBCM)) did not change

significantly. When the Mg^{2+} concentration increased from 0 to 900 mg/L, the concentrations of THMs and TBM decreased from 191 and 186.4 to 85.6 and 74.3 μg/L, respectively. Except for a small amount of TCM increasing from 1.7 to 7.9 μg/L, the concentrations of BDCM and DBCM did not change significantly. The results showed that the presence of Ca^{2+} and Mg^{2+} significantly reduced the formation of THMs during the chlorination process. This may be because Ca^{2+} and Mg^{2+} combine with the functional groups of HA to form chelates or complexes, thereby reducing the reaction between HA and chlorine. There are great differences in the research on the effect of hardness on the formation of DBP. The differences in the research results may be caused by the differences in DBP precursor species, temperature, pH, interfering ions and other factors, so further research is needed in the future. Whether or not Ca^{2+} and Mg^{2+} promote the formation of DBPs, their health benefits should not be ignored and their removal should not be encouraged. Other methods of controlling DBPs should be used.

Ng *et al.* (2015) found that bacterial cells (6.1–37.6 μg-THM/mg-C) were less capable of producing THMs than traditional DBP precursors such as HA and FA (40–140 μg-THM/mg-C). The bacterial concentration in source water is generally 10^4–10^5 cells/mL. According to the ability of bacterial cells to form DBPs (THM Formation = 22.4 μg-THM/mg-C, HAN formation = 6.1 μg-HAN/mg-C, CHD formation = 1.1 μg-CHD/mg-C), the concentration range of DBPs formed by bacterial cells during chlorination is 10–100 ng-THM/L, 3–30 ng-HAN/L, and 0.5–5 ng-CHD/L, respectively. If the concentration of DOM such as HA in the source water is 2–4 mg/L, the concentration ranges of DBPs are 2–45 μg-THM/L, 0.1–2.2 μg-HAN/L, and 0–2.6 μg-CHD/L, respectively. This suggests that NOM produces more DBPs than bacterial cells. In the process of wastewater treatment or medical water disinfection, BOM may generate more DBPs, because the bacterial concentration in the source water may reach 10^7–10^8 cells/mL. This suggests that the contribution of BOM to DBPs depends on the bacterial concentration in the raw water. The addition of HA to *E. coli* suspensions leads to a reduction in the concentration of effective chlorine used to inactivate the bacteria, and the presence of NOM produces a protective effect on the bacteria and reduces disinfection efficiency. In some cases, DBP produced is less when HA and *E. coli* coexist than that when HA is present alone. This may be due to the competition between HA and bacteria for chlorine during the chlorination process. However, this does not explain what happens when there is an excess of chlorine. This phenomenon may be attributed to the consumption of NOM by bacteria and the relatively low capacity of bacteria to form DBPs.

Besides NOM, AOM is also an important DBP precursor in source water. During the low rainfall season, AOM released by various microalgae accounted for 50% of the total DOC in surface water (Liu & Hong 2020). AOM contains various organic matters such as proteins, lipids, and amino acids, which are important DBP precursors. Hua *et al.* (2019) summarized the formation potential of THMs and HAAs of different algae at different growth stages and found that the range of THMFP of algae was 5–64 μg-THM/mg-C, and the range of HAAFP was 11–164 μg- HAA/mg-C. The concentration ranges of THMs and HAAs formed by algae were 16–98 μg-THM/L and 6–209 μg-HAA/L, respectively. From the common data, the order of THMFP is: NOM > AOM > BOM, and the order of THM production is: AOM > NOM > BOM. Although the contribution of different organics to DBPs varies with source water quality, based on the available data, the ability of BOM to generate THMs is the lowest. Even so, BOM cannot be ignored as an important source of N-DBPs. Overall, NOM and AOM might have stronger DBP-forming ability and could act as a nutrient source to enhance DBPFP of bacterial material. Therefore, the water treatment process should minimize the concentration of organic matter in the water.

4. CONCLUSION AND OUTLOOK

(1) Bacterial DBPs in drinking water distribution systems cannot be ignored. Bacterial materials can react with chlor(am)ine in the pipe network to generate C-DBPs and more toxic N-DBPs, posing a huge risk to drinking water safety. Among the main components of biofilms, proteins or amino acids have a greater risk of DBPs than polysaccharides and organic matter. Therefore, the removal of tube wall biofilm needs to be paid enough attention. The pipe network and its dead-end areas should be hydraulically flushed regularly to continuously ensure the safety of drinking water.

(2) Chlorine disinfection has greater advantages than chloramine disinfection in reducing biofilm and bacterial DBPs, but chlorine dioxide may be a better alternative. Most of the existing large water distribution pipelines are made of iron pipes. The risk of microorganisms and DBPs caused by corrosion of iron pipes should be paid attention. Pipe materials with better biological stability should be encouraged to use in order to reduce the generation of bacterial DBPs. PE pipe is the best choice for water supply pipeline. The concentration of organic matter and bromide ions in the water should be reduced as much as possible and the high flow rate of the water distribution system should be maintained within a reasonable range, so as to reduce the nutrient source for microbial growth and the production of DBPs.

(3) In order to control bacterial DBPs more efficiently, it is necessary to further explore the deep mechanism of the formation of bacterial DBPs in the future. The control, removal and monitoring devices of pipe wall biofilm need to be further developed and promoted to maintain the biological and chemical stability of the pipe network. Chlorine dioxide exhibits high biofilm removal capacity and low toxicity. If cost and storage issues are addressed, it may become a better alternative to chlorine and chloramines in the future. In order to reduce the risk of DBPs in the pipeline network, it is urgent to develop new disinfectants that are not easy to react with organic matter, and produce little or no DBPs, such as peracetic acid, potassium monopersulfate, potassium ferrate, tea polyphenols, etc. In recent years, they have attracted widespread attention from researchers due to their good bactericidal effect and low DBP generation. However, the large-scale application of these new disinfectants still needs to solve many problems, such as the improvement of basic research and regulatory standards, and the reduction of disinfection costs. Nevertheless, the development and promotion of new disinfectants is still an important means to reduce the risk of DBPs in drinking water and protect human health.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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