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MINI-REVIEW

Bacteriology of drinking water distribution systems: an integral and multidimensional review

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Abstract A drinking water distribution system (DWDS) is the final and essential step to supply safe and high-quality drinking water to customers. Biological processes, such as biofilm formation and detachment, microbial growth in bulk water, and the formation of loose deposits, may occur. These processes will lead to deterioration of the water quality during distribution. In extreme conditions, pathogens and opportunistic pathogens may proliferate and pose a health risk to consumers. It is, therefore, necessary to understand the bacteriology of DWDSs to develop effective strategies that can ensure the water quality at consumers' taps. The bacteriology of DWDSs, both the quantitative growth and the qualitative bacterial community, has attracted considerable research attention. However, the researchers have focused mainly on the pipe wall biofilm. In this review, DWDS bacteriology has been reviewed multidimensionally, including both the bacterial quantification and identification. For the first time, the available literature was reviewed with an emphasis on the subdivision of DWDS into four phases: bulk water, suspended solids, loose deposits, and pipe wall biofilm. Special concentration has been given to potential contribution of particulate matter: suspended particles and loose deposits. Two highlighted questions were reviewed and discussed: (1) where does most of the growth occur? And (2) what is the contribution of particle-associated bacteria to DWDS bacteriology and ecology? At the end of this review, recommendations were given based on the conclusion of this review to better understand the integral DWDS bacteriology.

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Introduction: drinking water distribution systems

A large amount of research has been conducted on water treatment, contributing to improved, treated water quality. The well-treated drinking water is delivered to customers' taps via distribution systems. There is a broad consensus that the final goal of water utilities should be to offer good quality drinking water at the customers' taps rather than only at the treatment plant. These drinking water distribution systems (DWDSs) should act as protective barriers and need to be operated and maintained to prevent contamination and growth of microorganisms as the treated water travels to the customer.

Treated drinking water enters the distribution system with a physical (particles) load, a microbial load (live biomass), and a nutrient load (biomass and nutrients, Fig. 1) (Liu et al. 2013a). As a result of biological and physiochemical processes during drinking water distribution, water at the consumers' taps has, in general, a lower quality than the treated water at the treatment plant (Lee et al. 1980; Hoehn 1988; Jones and Tuckwell 1993; Wable and Levi 1996; Matsui et al. 2007; Verberk et al. 2007; Vreeburg and Boxall 2007; Liu et al. 2013b). Many problems in DWDSs are caused by microorganisms, such as pipe wall biofilm growth (Camper 2004), nitrification (Regan et al. 2002; Regan et al. 2003), bio-corrosion of pipe material (Lee et al. 1980; Beech and Sunner 2004), deterioration of taste and odor (Hoehn 1988), and proliferation of opportunistic pathogenic bacteria (Emtiazi et al. 2004; Feazel et al. 2009). An understanding of the microbial ecology of distribution systems is necessary to design innovative and effective control strategies that will ensure safe and high-quality drinking water at the end tap (Berry et al. 2006).

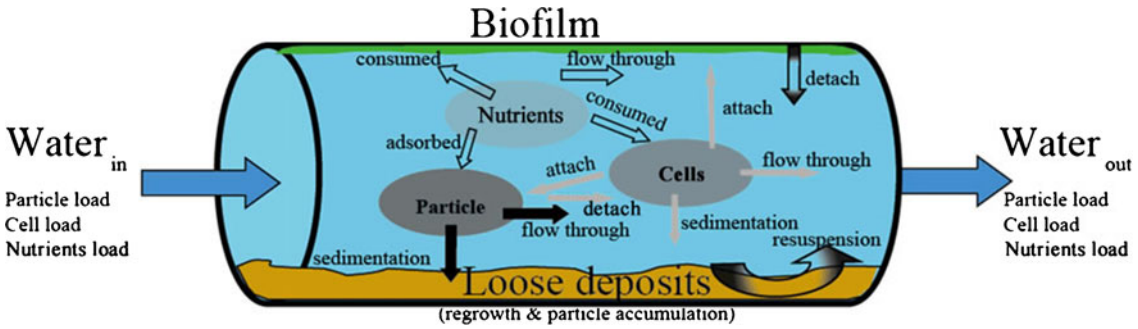

Fig. 1 Processes related to microbial growth during drinking water distribution

To understand its bacteriology, it is important to understand the microenvironments available in DWDSs. Based on the numerous studies on DWDSs and the different characteristics of each phase in the distribution system, the phases in DWDSs can be summarized as bulk water, suspended solids, pipe wall biofilm, and loose deposits. Bulk water is considered a transmission vehicle for nutrients, microbes, and particles throughout the distribution system. As the bulk water flows through the water main, a pipe wall biofilm forms on the inner surface of the pipe. The particles are transported throughout the network as colloids and suspended solids (SS) or may accumulate/settle as loose deposits on the pipe bottom. In practice, it is difficult to discriminate between colloids and suspended solids. When samples are taken by filtration over 0.5 μm or larger, most colloids will pass the filter, only suspended matter is measured. As schematically presented in Fig. 1, SS are the part of the particulate material that travels through distribution systems reaching the customers' taps under standard hydraulic conditions (Gauthier et al. 2001; Matsui et al. 2007). Loose deposits are the part of the particulate matter that settles at the bottom of the pipe under standard hydraulic conditions. During sudden hydraulic changes such as firefighting actions, flushing, or pipe breaks, loose deposits may be resuspended and become suspended solids (Gauthier et al. 1999; Zacheus et al. 2001; Lehtola et al. 2004c). Physiochemical processes in DWDS may have influences to more than one phase. For example, the corrosion of cast iron pipes releases particles and increases turbidity (Vreeburg et al. 2009), and at the same time, the formed incrustation favors the formation of pipe wall biofilm (Sarin et al. 2004).

Some reviews have been published on biological water quality in DWDS. However, each had a limited focus on a specific aspect and/or phase, and most of which focused on pipe wall biofilm: its impact on water quality, communities, influence factors, and control strategies (Batté et al. 2003a; Bachmann and Edyvean 2005; Berry et al. 2006; Chaves Simoes and Simoes 2013). Some reviews also covered the phase of bulk water, such as biological stability (van der Kooij 2000), heterotrophic bacteria (Chowdhury 2012), and mycobacteria (Vaerwijck et al. 2005) in DWDS. Although particulate matter also gained research attention, there is no integral concept including its contribution into DWDS bacteriology. Only one article was found with focus on the physical aspect of particulate matter: discoloration phenomenon (Vreeburg and Boxall 2007). In this article, DWDS bacteriology has been reviewed multidimensionally, including both the bacterial quantification and identification. The available literature was reviewed with an emphasis on the subdivision of DWDS into four phases: bulk water, suspended solids, loose deposits, and pipe wall biofilm. Special attention has been given to the potential contribution of particulate matter, suspended particles, and loose deposits on DWDS bacteriology.

Quantification of microbial growth in DWDSs

Microbial growth can be observed by the increase of biological activities or cell numbers occurring during drinking water distribution. Brazos and O'Connor (1985) proposed specific definitions for two terms, used synonymously, to describe the occurrence of blooms or high bacterial populations in drinking water distribution systems: “regrowth” and “after growth.” Regrowth refers to the recovery of the cells (possibly disinfectant injured and/or other unfavorable conditions), which have entered the distribution system from the water source or treatment plant, while after growth is the growth of microbes native to a water distribution system. In this review, the universal term of microbial growth (in short: growth) will be used to cover any kind of growth. The first step needed to understand and evaluate growth is to quantify the growth. For understanding the literature on growth during drinking water distribution, it is important to understand the methods used to study it. So, before discussing the growth in different phases in the water distribution network, the methods for such a study will be discussed in the following paragraphs.

Biological stability

Definition of biological stability

The concept and definition of biological stability have been proposed (Rittmann and Snoeyink 1984) and developed

(Miettinen et al. 1997; Van Der Kooij 2000; Srinivasan and Harrington 2007; Hammes et al. 2010a) over many years. In short, it is agreed that biological stability is a concept that addresses the overall tendency of water (and/or contact material) to promote microbial growth. Biological stability is measured as the level of growth supported by water or material contacted with water. Water is more biologically stable if fewer nutrients are present in the water (Table 1). Different factors were described by researchers regarding the impact of the presence (Srinivasan et al. 2008) or absence of a disinfectant (Rittmann and Snoeyink 1984), the importance of the phases of bulk water only or both water and contacted materials (Rittmann and Snoeyink 1984; Van Der Kooij 2000), and the dependence on organic nutrients (Van Der Kooij 2000; Escobar and Randall 2001) or inorganic substances (e.g., phosphate) (Kerneïs et al. 1995; Miettinen et al. 1997). Most of the researches have been focused on and limited to bulk water and pipe wall biofilm. There is a knowledge gap in the attention to the association of suspended solids and loose deposits.

Guidelines for biological stability

Different methods were used to determine biological stability, such as assimilable organic carbon (AOC) (Van der Kooij et al. 1982) and biodegradable dissolved organic carbon (BDOC) (Servais et al. 1987), both of which represent the fraction of dissolved organic carbon that may readily support growth of microbes.

AOC was first proposed as a measurement of the organic content available for bacterial growth by Van der Kooij et al. (1982) and further modified by others (Kemmy et al. 1989; FrÃas et al. 1994; Hammes and Egli 2005; Zhang et al. 2007). The method evaluates the growth supporting organic carbon by quantifying the growth of selected bacteria and transferring the amount of bacteria back to nutrient concentration. It has been widely reported that an increase in AOC in treated water stimulates the growth of bacteria in both bulk solution and biofilms of DWDS (van der Kooij et al. 1995; Lechevallier et al. 1996; Tsai et al. 2004; Hu et al. 2005).

BDOC represents the biodegradable fraction of dissolved organic carbon (DOC) (Ollos et al. 2003; Ndiongue et al. 2005). It is quantified by measuring the DOC reduction caused by growth of naturally occurring bacteria. Most studies showed a positive relationship between the BDOC concentration and bacterial growth in DWDS (Van der Kooij et al. 1982; Owen et al. 1995; Lu et al. 1999; Volk and LeChevallier 1999; Ndiongue et al. 2005), but some showed either a weak correlation (Camper et al. 1996) or no correlation at all (Escobar et al. 2001).

Although AOC and BDOC have been well studied, commonly used, and have guidelines (AOC/BDOC levels at which the water is sufficiently stable/limits growth in water, Table 1) that have been established, the two methods still have limitations. For example, the methods are indirect and quantify the available nutrients instead of quantifying bacteria directly, both methods assume growth is limited by the organic carbon source (Van Der Kooij 2000), the methods depend on the type of bacteria used (Hammes and Egli 2005), and they are also time consuming and expensive to perform. Considering the multiple phases in DWDS, AOC and BDOC are used primarily to determine readily biodegradable organic nutrient levels in the bulk water. In another word, the possible contribution of nutrients and biomass hosted by loose deposits has been totally neglected.

Direct measurement of bacterial concentration

Bacterial concentration (biomass) quantification is the most direct way to monitor growth. One of the more contentious aspects of the growth studies is the choice of appropriate microbial parameters to evaluate it (Hammes et al. 2010a).

HPC

Heterotrophic plate counts (HPCs) have been used since 1894 to determine bacterial concentrations in distributed drinking water (Bartram 2003). HPC is the primary parameter for assessment of the general microbiological quality of drinking water (Chowdhury 2012). Bulk water, loose deposit bacteria, and pipe wall biofilm bacteria have all been quantified by HPC, with a reported value range of 0–10^3 CFU ml^{-1} for bulk water, 10^6–10^8 CFU cm^{-2} for pipe wall biofilm bacteria, and 10^8 CFU g^{-1} for loose deposit bacteria (Table 2). Since

Table 1 Reported biological stability guidelines for AOC and BDOC

	Reported value	Literature	Biological stability guideline value	Literature
AOC	1.9–400 μg l^{-1}	Van der Kooij (1992), Kaplan et al. (1994), Miettinen et al. (1997), Volk and LeChevallier (2000), Liu et al. (2002), Zhang et al. (2007), Van der Wielen and Van der Kooij (2010)	10 μg C l^{-1} (no chlorine, the Netherlands)	Van der Kooij (1992)
			50–100 μg C l^{-1} (with chlorine, USA)	LeChevallier et al. (1987)
BDOC	<0.1–1 mg l^{-1}, typically 0.24–0.32 mg l^{-1}	Kaplan et al. (1994), Volk and LeChevallier (1999), Bachmann and Edyvean (2005)	150 μg C l^{-1}	Servais et al. (1995)

Table 2 Concentration of bacteria in different phases in the network

Phase	Quantity (as determined with different methods)				References
	HPC	TCC	ATP	Percentage of biofilm bacteria	
Pipe wall biofilm	10^8–10^{10} CFU ml^{-1}				Van Der Wende et al. (1989)
	10^6–10^8 CFU cm^{-2}				Batté et al. (2003a)
	10^7 CFU cm^{-2}	10^7 cells cm^{-2}	100 pg cm^{-2}		Lehtola et al. (2004b)
	2.6×10^6 CFU cm^{-2}	1.3×10^7 cells cm^{-2}	4,056 pg cm^{-2}		Lehtola et al. (2006)
			500 pg cm^{-2}		Yu et al. (2010)
Bulk water	0–4,500 CFU ml^{-1}	0.4–8.0×10^{5}cells ml^{-1}	0.32–28 ng l^{-1}		Van der Wielen and Van der Kooij (2010)
	5 CFU ml^{-1}	0.9×10^5cells ml^{-1}	16–55 ng l^{-1}		Hammes et al. (2010a)
	10^2–10^5 CFU ml^{-1}				Van Der Wende et al. (1989)
	0.3–556.3 CFU ml^{-1}	1.3–2.6×10^5 cells ml^{-1}			Henne et al. (2012)
Loose deposits	2.5×10^8 CFU g^{-1}				Gauthier et al. (1999)
	10^5 CFU ml^{-1}	10^8 cells ml^{-1}			Zacheus et al. (2001)
	2.2×10^8 CFU g^{-1}				Torvinen et al. (2004)
Bulk water bacteria (BWB) vs. pipe wall biofilm bacteria (PWBB)	BWB[a], 2.40×10^7 CFU; PWBB[a], 1.86×10^7 CFU	BWB, 2.10×10^9 cells; PWBB, 4.01×10^9 cells	BWB, 6.5×10^6 pg; PWBB, 4.34×10^7 pg		Boe-Hansen et al. (2002)
	BWB, 2.00×10^7 CFU; PWBB, 4×10^5 CFU		BWB, 5×10^3 pg; PWBB, 3.5×10^4 pg	0.1–9.0 % (no chlorine)	Silhan et al. (2006)
	BWB, 2.23×10^8 CFU; PWBB, 2.00×10^7 CFU			17–35 % (0.2 mg l^{-1} chlorine)	Manuel et al. (2007)
				19 % (no chlorine), 63 % (0.2 mg l^{-1} chlorine), 70 % (0.5 mg l^{-1} chlorine)	Srinivasan and Harrington (2007)

[a] SB and pipe wall biofilm bacteria are calculated according to methods proposed by Srinivasan et al. (2008)

different units were used to quantify the bacteria of different phases, it is difficult to compare the obtained HPC values. In order to compare the contribution of different phases, total HPC numbers were calculated based on the water volume, the pipe surface area, and the amount of the mass of loose deposit described in the literature and a biomass balance was made (Srinivasan et al. 2008). Different from the previous conclusion that pipe wall biofilm contributes most to the biomass, bulk water was found to contain more HPC than pipe wall biofilm.

The most critical limitation of HPC is that it only counts media-cultivable bacteria where less than 20 % of the total cells can be detected by this culture-based method, and the fraction of cells which are not cultivable under standard culture conditions is not recognized in aquatic environments (Ford 1999; Lehtola et al. 2006; Manuel et al. 2007). In drinking water, the percentage may decrease between 0.001 and 6.5 % (Hammes et al. 2008, Berney et al. 2008). Moreover, different nutrients, culture media, temperature, and incubation periods applied in HPC methods result in significant differences in HPC enumeration (Reasoner 2004) and difficulties in comparing the results.

Total cell count

To overcome the disadvantage of HPC and get information about the total number of bacteria present, total cell count (TCC) can be used. The total cell count determines bacteria numbers by concentrating bacteria using membrane filtration and staining the bacteria with a fluorescent dye (i.e., acridine orange), followed by microscopic counting (Boe-Hansen et al. 2002). For optimal accuracy, it is recommended to count about 400 cells on multiple filters, making this method both time consuming and subjective (Šantić et al. 2007). The reported TCC results are 10^5 to 10^7 cells ml^{-1} in bulk water, 10^5 to 10^7 cells cm^{-2} on pipe wall biofilm, and 10^8 cells ml^{-1} for loose deposits (Table 2). The biomass balance calculation confirmed the results of HPC: suspended bacteria contribute

more to the total bacteria numbers in the network than pipe wall biofilm bacteria.

The flow cytometry method (FCM) for a total bacteria count was recently introduced in drinking water research. FCM has been found to be rapid and simple (Berney et al. 2008). It can count both the cultivable and uncultivable cells with high sensitivity and accuracy (200 cells ml^{-1}, std. below 5 %) (Sklar 2005; Hammes et al. 2010a). Research has been conducted to compare the direct count and flow cytometry methods, from which FCM was reported to be more accurate (Šantić et al. 2007). The use of FCM in the field of drinking water distribution system research is still limited. It has been reported that in drinking water distribution systems without disinfectant residuals, total cell count for bulk water is around 10^5 cells ml^{-1} (Berney et al. 2008; Hammes et al. 2010a; Liu et al. 2013b). There are, to date, no FCM results of pipe wall biofilm bacteria or loose deposits bacteria available.

Adenosine triphosphate

The adenosine triphosphate (ATP) assay is a rapid approach with low detection limits (as low as 0.0001 nM, <5 % deviation). The method determines all biologically active bacteria through ATP measurement (Hammes et al. 2010b; Van der Wielen and Van der Kooij 2010; Liu et al. 2013b). A strong relationship between FCM-DC and ATP concentration was observed in drinking water samples (Berney et al. 2008; Siebel et al. 2008; Hammes et al. 2010b). ATP has been suggested as a suitable and fast method for screening and detecting growth since high ATP concentrations correlate with high *Aeromonas* numbers (Van der Wielen and Van der Kooij 2010).

ATP has been widely applied to quantify active biomass in water treatment processes (Magic-Knezev and van der Kooij 2004; Velten et al. 2007; Berney et al. 2008), distributed water (Hammes et al. 2010b; Van der Wielen and Van der Kooij 2010), and pipe wall biofilm bacteria in drinking water distribution systems (Boe-Hansen et al. 2003, Martiny et al. 2003; Delahaye et al. 2003; Lehtola et al. 2004a). The ATP in unchlorinated bulk water was reported between 0.32 and 28 ng l^{-1} in the Netherlands and 16 and 55 ng l^{-1} in Switzerland. With a bulk water ATP of 25–311 ng l^{-1}, pipe wall biofilm bacteria in the same system contained ATP ranging from 0.07 to 4 ng cm^{-2}. The calculated total ATP (based on water volume and surface area of the pipe wall) is comparable in bulk water and pipe wall biofilm bacteria. There is still no research on loose deposits ATP in DWDS available.

It should be noted that all mentioned methods are designed for water samples. For measurements conducted with surface-attached samples, pretreatment is necessary to detach the microbes into suspension for further analysis. Moreover, no single method can determine all relevant aspects, so it is necessary to combine the information obtained by the different methods. It is generally agreed that a combination of methods that focuses on different indicators of viability is superior to any individual method, especially when natural microbial communities are being assessed (Berney et al. 2008). By combining cell count and cell activity, not only can the number of cells and the activity of the cell be studied, but the physiological state of the cell can also be assessed (Berney et al. 2008; Hammes et al. 2010b).

Fractions of biomass in DWDS: where does most of the growth occur?

Although the above-mentioned studies suggest that microbial growth may occur in every phase of the attached pipe wall biofilm, the bulk water (WA), the SS, and loose deposits, the essential question of where most of the growth is occurring is still unknown. There was a common notion that over 90 % of biomass was present on the pipe wall biofilm. Contributions from other phases have been considered to be negligible (LeChevallier et al. 1987; Flemming et al. 2002; Lehtola et al. 2004b; Servais et al. 2004). In oligotrophic environments, and in the presence of disinfectant residuals in DWDS (not applied in the Netherlands), various microbes survived and grew. Examples of those include the pathogenic microorganisms of *Legionella*, *Aeromonas* spp., and *Mycobacterium* spp. (Emtiazi et al. 2004); autotrophic bacteria of *Nitrospira* (Hoefel et al. 2005); and heterotrophic bacteria of *Planctomyces*, *Acidobacterium*, and *Pseudomonas* (Martiny et al. 2003; Williams et al. 2004; Eichler et al. 2006; Srinivasan et al. 2008). The survival and growth of microbes have been attributed to the favorable conditions offered by the pipe wall biofilm (Stewart et al. 1996) and the protection offered by the surrounding matrix of extracellular polymeric substances (EPS) (Allesen-Holm et al. 2006). Consequently, most of the reported studies and reviews have focused on and limited to pipe wall biofilm bacteria.

The microbial growth in DWDS has also been reported in DWDS loose deposits. Loose deposits attracted research interests mainly because of the corrosion process, the accumulation of inorganic contaminants (Peng et al. 2010), and the phenomenon of discoloration (Vreeburg and Boxall 2007). Suspended particles/loose deposits were considered possible factors that might enhance biological growth (Vreeburg et al. 2008), either by supplying nutrients, offering surface area, or protecting bacteria from disinfectants, if applicable (Gauthier et al. 1999). The investigations of loose deposits found that loose deposits are reservoirs for organic carbon and bacteria (Gauthier et al. 1999; Zacheus et al. 2001; Liu et al. 2013a). However, the contribution of loose deposits has been neglected in the previous evaluation of the overall growth during drinking water distribution.

Little research has been done that covers multiple phases in the same study. One such study, comparing the growth of pipe

wall biofilm bacteria and bacteria in bulk water, has revealed that the bacterial growth rate in the biofilm is lower than that in the bulk water (Boe-Hansen et al. 2002). Comparing the biomass in the bulk water and the biofilm, it was found that biofilms that attached to the surface pipe contained 25 times more bacterial cells than in the bulk water per unit pipe length (Servais et al. 2004).

However, none of these studies have assessed/compared the contributions of the different phases to the overall microbial growth. Historical data from different studies were reviewed (Table 2), but it is difficult to make the comparison across different phases within DWDS from these data. The difficulties are partly caused by the differences between systems, no study addressed all phases in a single system, and partly caused by the use of different quantification methods and different units to present the results. For example, HPC, DC/FCM, and ATP are three methods that are widely used to quantify bacteria in drinking water distribution systems, and pipe wall biofilm bacteria is commonly represented as the number of bacteria per surface area (in square centimeter), bulk water bacteria were quantified as the number of bacteria per volume of water (in milliliter), and the bacteria in loose deposits were quantified as the number of bacteria per mass deposits (in gram).

As mentioned above, a calculation of total bacteria and biomass balance can be made if information on surface area, volume of water, and mass deposits were given in the study (Srinivasan et al. 2008). Taking the cultivable bacteria in deposits to be 2.5×10^5 CFU g^{-1} (Gauthier et al. 1999), assuming that the average concentration of cultivable bacteria in the biofilm is 10^5 CFU cm^{-2}, Batté et al. (2003a) came to the conclusion that if there is more than 1 g m^{-2} of loose deposits accumulated in the DWDS, more bacteria will be associated with loose deposits than with the pipe wall biofilm. When more than 10 g m^{-2} of loose deposits has accumulated, loose deposits bacteria may represent more than 80 % of the total bacteria in DWDSs (Fig. 2). The reported loose deposits values are commonly close to or even higher than these concentrations (Zacheus et al. 2001). However, this is, until now, still based on assumptions and needs to be proven in field distribution systems. It should be noted that the values of loose deposits bacteria were close to those that have been reported in the literature, whereas the assumption of pipe wall biofilm as 10^5 CFU cm^{-2} is lower than the reported values (10^6–10^8 CFU cm^{-2}).

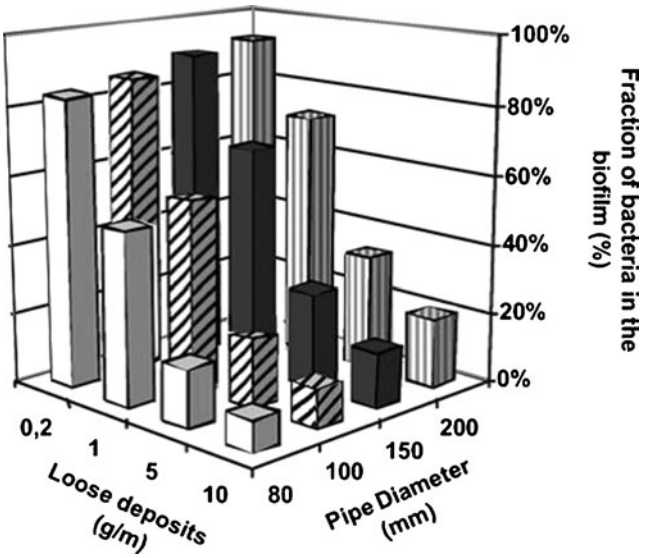

Fig. 2 Comparison of biofilm and loose deposit fractions of cultivable bacteria in a 1-m pipe section based on the literature with a hypothesized average concentration of cultivable bacteria in the biofilm of 10^5 CFU cm^{-2} (Batté et al. 2003a)

Bacterial identification: community analysis

Data on the microbial community diversity of DWDSs are far from being thoroughly assessed and understood, as the molecular tools have not yet been used widely in this field (Berry et al. 2006; Mathieu et al. 2009). Overall, previous studies have investigated bacterial abundance, community diversity, and composition at different points (Eichler et al. 2006; Pinto et al. 2012), scales (Williams et al. 2005; Peng et al. 2010), and phases of DWDSs (Norton and LeChevallier 2000; Henne et al. 2012). Predominant phyla and genera, commonly encountered pathogens, and opportunistic pathogens are summarized from these studies in Table 3. It should be noticed that the use of different detection methods may influence the fraction of pathogens that can be detected. For example, only a small fraction of pathogens can be detected by culture-based detection method (e.g., clone library), culture-independent methods (e.g., PCR-DGGE) can detect more pathogens, and even more pathogens can be detected by the next-generation sequencing technique (e.g., 454 pyrosequencing).

Bacteria in bulk water and pipe wall biofilm

Most of the available information is on the pipe wall biofilm because, as mentioned above, it was believed that more than 95 % of the bacteria in DWDSs is in the pipe wall biofilm (Flemming et al. 2002; Servais et al. 2004), and pipe wall biofilm bacteria have a higher resistance to disinfectants (Emtiazi et al. 2004). The opportunities to sample pipe wall biofilm from field distribution systems have been and are limited, and the sampling is costly. As a result, many studies have used model distribution systems and/or sampling coupons for biofilm attachment in field distribution systems. A good example is the study of biofilm that formed on two water meters in the distribution system in Urbana-Champaign, IL, USA (Peng et al. 2010).

Table 3 Commonly found bacteria in DWDSs

	Commonly found bacteria in DWDSs	References
Predominant phyla in DWDSs	Proteobacteria, Actinobacteria, Firmicutes, Verrucomicrobia, Nitrospirae, Bacteroidetes	Kalmbach et al. (1997), Schwartz et al. (1998), Schmeisser et al. (2003), (Williams et al. 2004, 2005), Peng et al. (2010)
Predominant genera in DWDSs	*Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Arthrobacter*, *Corynebacterium*, *Bacillus*, *Burkholderia*, *Citrobacter*, *Enterobacter*, *Flavobacterium*, *Klebsiella*, *Methylobacterium*, *Moraxella*, *Pseudomonas*, *Seratia*, *Staphylococcus*, *Mycobacterium*, *Sphingomonas*, *Xanthomonas*	Batté et al. (2003a), Berry et al. (2006), Simões et al. (2010)
Opportunistic pathogens	*Mycobacterium*, *Legionella*, *Pseudomonas*, *Klebsiella*, and *Aeromonas*	WHO (2008)

Influencing factors

The bacterial community structure and diversity differed depending on the pipe materials, disinfection strategies, and age of the biofilm. For instance, the drinking water biofilms from Berlin's (Kalmbach et al. 1997; Schwartz et al. 1998) and Montreal's (Batté et al. 2003b) distribution systems were characterized by a high number of Betaproteobacteria; distribution system biofilms from Urbana-Champaign were characterized by high numbers of both Alpha- and Betaproteobacteria (Peng et al. 2010); and high numbers of both Alpha- and Gammaproteobacteria were found in the distribution system of a town located in North Rhine-Westphalia, Germany (Schmeisser et al. 2003).

Microbial richness and diversity can be influenced significantly by the pipe material (Donlan 2002; Yu et al. 2010). Especially for cast iron pipe, the released iron may promote growth of iron bacteria, and the formed corrosion scales can favor the formation and growth of biofilm (Sarin et al. 2004). Nevertheless, a study of mature biofilms in field distribution systems that had been used for 20 years found low dependency of the community structure on the surface material (Henne et al. 2012). The low dependency was attributed to the mutual influence of adjacent biofilms by the exchange of bacteria. Similarly, another long-term pilot study found that after 3 years, most of the biofilms from different sampling points showed a homogeneous community structure (Martiny et al. 2003). Based on these observations, Henne et al. (2012) hypothesized that after the surface had been colonized by surface-specific biofilm, an adjacent, year-long coexistence may lead to a mutual influence of biofilms at different sites in the network. Gradually, the biofilm would be overgrown by the nearby biofilm community, leading to a homogeneous biofilm throughout the network.

Disinfection strategies, both the type and dose of disinfectant, have significant influence on bacterial diversity (Berry et al. 2006). For example, Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria were the major groups in chlorinated systems (Tokajian et al. 2005). Alphaproteobacteria were the dominant groups in chloraminated model systems, whereas Betaproteobacteria were found to be more abundant in chloraminated systems than chlorinated systems (Williams et al. 2004). In the non-chlorinated system, Betaproteobacteria were the dominant groups (Emtiazi et al. 2004). There is also evidence that Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria have different sensitivities to chlorine, among which Gammaproteobacteria have the lowest sensitivity/highest resistance (Mathieu et al. 2009). The same study reported that the population shifts of biofilm exposed to discontinuous chlorination were reversible. In return, the diversity of the microbial community can affect the disinfection efficiency and pathogen survival. For example, multispecies biofilms showed higher resistance to biocides than single-species biofilms (Elvers et al. 2002).

Comparison of bulk water bacteria and pipe wall biofilm bacteria

Comparing the microbial population attached to pipe surfaces with the bacteria present in the bulk water, clear differences were observed in both a model system (Norton and LeChevallier 2000) and a field distribution system (Henne et al. 2012). It is noted that both of the studies were conducted in a distribution system with a chlorine residual, indicating the results may be influenced by the selective pressures on the bulk water bacteria posed by disinfection. On the other hand, as the bulk water bacteria are living freely suspended in the water and the pipe wall biofilm bacteria are growing on a support surface, the difference may also be attributed to the varying attachment capabilities of different groups of bacteria. If the conclusion that the majority of the biomass is in the biofilm and that bulk water bacteria mainly originate from pipe wall biofilm detachment is true, then the difference could also be related to the detachment properties of the biofilm bacteria. For example, only the top layers (loose structure) are readily released into bulk water, and the inner biofilm remains attached to the pipe wall.

As only bacteria community information of bulk water and pipe wall biofilm is available, it is not possible to make a comparison of bacteria from all four phases. It is therefore highly necessary to improve our understanding of the bacteriology of DWDSs. Possible contributions of particle-associated bacteria to DWDSs bacteriology will be discussed in the following section.

Importance of bacteria associated with suspended solids and loose deposits

Similar to the pipe wall, the particles in distribution systems offer surfaces for bacteria to attach to and to form biofilm (Winkelmann and Harder 2009). As the particles contain both organic and inorganic nutrients, they may function as adsorbents for attachment of bacteria (Gregory 2005). The major concern of these particle-associated bacteria is that the suspended solids/loose deposits can protect bacteria from disinfection by chlorine (Ridgway and Olson 1981), ozone (Hess-Erga et al. 2008), and ultraviolet light (Wu et al. 2005). Among the limited available information about loose deposit bacteria, pathogens and opportunistic pathogens have been reported, such as Mycobacteria (Torvinen et al. 2004). Considering the better mobility of particle-associated bacteria than pipe wall biofilm bacteria, the former may be transferable throughout distribution systems. During the hydraulic peaks, these particle-associated bacteria may even reach customers' taps and be consumed by customers. This can potentially lead to increased exposure in the case of particle-associated (opportunistic) pathogens.

Based on the similar properties of particle-associated bacteria and pipe wall biofilm bacteria, it is hypothesized that the bacterial community in suspended solids/loose deposits is similar to the pipe wall biofilm bacterial community. Depending on the characteristics of the particles, differences can also be expected. Moreover, it has been reported that as much as 24.5 g m^{-1} of loose deposits was found in the field distribution system (Carrière et al. 2005). The accumulation of loose deposits together with contained bacteria (combined with EPS) may create different microenvironments compared to bulk water and pipe wall biofilm, such as anoxic and/or sub-anoxic conditions. In short, it can be concluded that loose deposits bacteria are even more important than pipe wall biofilm.

Controlling microbial growth

Controlling microbial growth during drinking water distribution is difficult because of the complexity of distribution systems. On one hand, distribution systems are usually complex systems that are hundreds of thousands meters long with different and variable hydraulic conditions, different pipe materials, and feed water quality, which are usually underground. On the other hand, the survival and growth of microbes are complex processes that depend on the interactions of many variable factors, such as temperature, nutrients, water age, and types and concentrations of disinfectant residuals.

The two approaches used to control microbial growth are (1) providing disinfectant residuals and (2) producing biologically stable water (Van Der Kooij 2000). The use of disinfectant residuals, however, will lead to problems of disinfection by-products (Bull 1982) and deterioration of taste and odor (Bryan et al. 1973). Another limitation of using disinfectant residuals is the finding of disinfectant-resistant bacteria (Hoff and Akin 1986) and the protection of bacteria by biofilm or particles in the distribution system (Ridgway and Olson 1981; Emtiazi et al. 2004), which means that, in such distribution systems, these bacteria may grow without limitation when more than sufficient nutrients are available.

The approach of producing and supplying biologically stable water can limit the growth of any kinds of bacteria by controlling the food source (Van der Kooij 2003). The advantages of this approach are no formation of DBP and no influence on the taste and odor of the drinking water. Moreover, the growth of disinfectant-resistant bacteria and protected bacteria can also be efficiently controlled. Of course, this approach is highly dependent on the efficiency of the treatment processes to obtain nutrient concentrations low enough to sufficiently limit bacterial growth. The pipe material for water distribution should also be biologically stable. The stability can be measured as biomass production potential (Van der Kooij et al. 2006).

Within both approaches, the research on loose deposits has highlighted both the quantitative and qualitative importance of loose deposit bacteria. The accumulation of loose deposits and associated bacteria should be controlled by limiting the particle load fed to the distribution system (Vreeburg et al. 2008), and the formed loose deposits should be removed regularly by flushing the system, which has been proven to be an efficient way to reduce microbial growth and improve water quality (Lehtola et al. 2004c).

Table 4 Classification and characterization of phases in DWDSs

Phases	Characteristics	References
Bulk water	Transmission vehicle for nutrients, microbes, and particles; seed bank for microbial growth and particle accumulation	Boe-Hansen et al. (2002), Blackburn et al. (2004), Henne et al. (2012)
Suspended solids	Suspended in bulk water offers surface area for bacteria to attach and grow; provides protection and nutrients to the attached bacteria	Brazos and O'Connor (1996), Gauthier et al. (2001), Matsui et al. (2007)
Pipe wall biofilm	Accounts for major bacteria in DWDS; protects bacteria from disinfection	LeChevallier et al. (1987), Flemming et al. (2002), Emtiazi et al. (2004)
Loose deposits	Settled at the pipe bottom and transferrable to bulk water during hydraulic peaks; provides protection and nutrients to enriched bacteria	Gauthier et al. (1999), Zacheus et al. (2001), Lehtola et al. (2004c)

Recent studies offer more insight and possibilities for controlling the growth qualitatively. A study comparing bacterial communities in treatment trains and those in the distributed water found that drinking water microbiology is governed by the filtration steps applied in the drinking water treatment (Pinto et al. 2012). The finding presented a possible opportunity to control and manage the bacteriology at the treatment plant. Another study comparing bulk water bacteria and pipe wall biofilm hypothesized that the low-abundance bacteria from the bulk water function as a seed bank for the biofilm, indicating that the pipe wall biofilm population can be controlled by controlling the bulk water (Henne et al. 2012). A deeper understanding of the relationship between treatment processes, bulk water bacteria, and pipe wall biofilm will favor the effective management of the microbiological quality of distributed water.

Conclusions and recommendations

Over decades of research, drinking water distribution system bacteriology has been partially illuminated, both quantitatively and qualitatively. The contribution and importance of the different phases are summarized in Table 4. Based on the review of the literature, the main conclusions are outlined below:

1. Direct biomass quantification should be encouraged in future research work, and a combination of methods should be used instead of any single method (HPC, DC, and ATP).
2. Microbial growth occurs in different/all phases. The direct comparison of each phase is not available. It was previously believed that most of the growth occurs in the pipe wall biofilm. However, particulate matter has also a potential importance for the growth, and high numbers of bacteria have been found in loose deposits.
3. The understanding of bacterial community structure and composition is limited overall, and the available knowledge is mainly limited to the phases of the pipe wall biofilm, though some research did look into bulk water. The bacterial community of suspended solids bacteria and loose deposits bacteria is still unknown, neither do their contribution to DWDS bacteriology.

Clearly, we notice that the bacteriology of drinking water distribution systems is still poorly understood regarding the important research questions of where does most of the growth occur, what is the community of bacteria associated with suspended particles and loose deposits, and what is their contribution to the bacteriology and microbial ecology of DWDSs. Further integral research cover all of the four phases is needed, especially for the suspended particles and loose deposits, where considerable amount of bacteria and different communities can be expected.

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