Biofilm in the Parisian suburbs drinking water distribution system

Pierre Servais, Adriana Anzil, Dominique Gatel and Jacques Cavard

ABSTRACT

Internal pipe surfaces within drinking water distribution systems (DS) are colonised by bacteria forming a biofilm. Detachment of cells from the biofilm leads to a deterioration of the microbiological water quality. This justifies the requirement for biofilm investigation in DS. This paper presents data on biofilm gained over a long period in the different areas of the Parisian suburbs DS. Two different systems were tested to incubate cast-iron coupons in DS to measure biofilm after colonisation in order to study biofilm. It shows that a simple and inexpensive system can be efficiently used for this purpose. Fixed bacterial biomass was routinely estimated, in this study, on cast-iron coupons by potential exoproteolytic activity (PEPA) and sometimes by epifluorescence microscopic enumeration performed after bacteria detachment from the support by sonication. Bacterial biomass in the biofilm ranged from 0.009 to 0.56 µgC cm^{-2} in the studied distribution systems. Comparing bacterial numbers in the water phase, on one hand, and fixed to pipe walls, on the other hand, showed that fixed cells were much more abundant than the suspended cells in DS (25 times in a 100 mm diameter pipe). The large set of data gained on biofilm within the Parisian suburbs DS allows us to demonstrate, on full scale data, the major role of disinfectant (chlorine) residual and biodegradable dissolved organic carbon (BDOC) as controlling factors of the biofilm in DS.

Key words | bacteria, biodegradable dissolved organic carbon, biofilm, chlorine, distribution system, drinking water

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INTRODUCTION

There is now clear evidence that all internal pipe surfaces within drinking water distribution systems (DS) are colonised by bacteria (Rigway & Olson 1981; LeChevallier *et al*. 1987; Block 1992; van der Kooij *et al*. 1995). These bacteria are fixed onto the internal wall of the pipes by exopolymers composed of proteins and polysaccharides and form non-continuous thin layers called biofilm. When bacteria in the biofilm are growing or when shear stress occurs, release of cells from the biofilm to the water phase may occur, increasing bacterial density in the water phase and decreasing the microbiological quality of the distributed water. Thus, bacteria forming a biofilm in drinking water DS can be the cause of multiple problems of quality: bacteria can be the starting point of a trophic food web leading to the occurrence of undesirable higher organisms (protozoa, invertebrates, crustaceans, etc.) (Lévy *et al*. 1986); the occurrence of some species can generate turbidity, taste and odours (Mallevialle & Suffet 1987); accumulation of bacteria can promote corrosion (Emde *et al*. 1992); high numbers of bacteria can interfere with the detection of coliforms or other sanitary indicators (LeChevallier 1990).

Even if the importance of controlling biofilm development in DS to maintain water quality is well recognised, the quantification and study of biofilm in full scale DS is not an easy thing. It is the reason why numerous studies on the factors controlling biofilm development were conducted on various types of pilots. A large number of

bench scale systems and industrial pilots have been described in the literature. In the former, annular reactors (Rototorque™ (Van der Wende *et al.* 1989) and Propella™ (Appenzeller *et al.* 2001)), chemostats, in which the residence time is independent from the shear stress which is fixed by the rotational speed of the system, were developed and extensively used. Two main types of industrial pilot were designed: the pipe loop distribution system (Haudidier *et al.* 1988) and the open pipe rigs (Holt *et al.* 1994). However these pilots cannot really reflect all the complexity and the heterogeneity of a full scale DS; that is why some authors have tried to investigate the biofilm directly on pipe sections removed from full scale DS. This was a cumbersome and expensive approach that required an area within the system to be shut down to remove a piece of pipe (Rigway & Olson 1981). Others developed sample devices allowing coupons (Servais *et al.* 1992*a*) or cylinders (Donlan & Pipes 1988) to be inserted in and removed from pressurised water mains to quantify attached micro-organisms. Finally, some authors used incubation devices fed continuously with water from the DS that allow the colonisation of pieces of material in conditions mimicking those met in DS (Servais *et al.* 1995; van der Kooij *et al.* 1995; Niquette *et al.* 2001) with subsequent investigation of the biofilm.

In addition to the differences described above between the methods used to obtain biofilm to study, the analytical methods were also quite different from one study to another. Most of the authors first detach bacteria from the pipes by sonication (Mathieu *et al.* 1992) or scraping (Le Chevallier *et al.* 1987). Once detached, the bacteria can be enumerated by classical plate count (on R2A medium, for example), by epifluorescence microscopy after acridine orange or DAPI staining (Mathieu *et al.* 1992) or by biomass estimation through ATP analysis (van der Kooij *et al.* 1995). Others used methods that do not need bacteria detachment such as the measurement of potential exoproteolytic activity (Laurent & Servais 1995; Butterfield *et al.* 2002). Thus, the large variety of experimental approaches to quantify drinking water biofilms (colonisation and analytical procedures), added to the fact that the different investigators studied biofilm fixed on various materials, makes the comparison of data obtained by different studies extremely difficult.

The aim of this paper is first to discuss some technical aspects of the methods for quantifying biofilm in full scale DS on the basis of data gained in the Parisian suburbs DS. In addition, the main factors controlling the density of biofilm in full scale DS will be discussed using a large database collected for more than 10 years on the different sectors of Parisian suburbs DS.

MATERIAL AND METHODS

The Parisian suburbs treatment plants and DS

The present study was conducted on the DS of the Parisian suburbs which supplies drinking water to approximately 4 million people in 144 municipalities. This DS is composed of three sectors, each one being fed by a treatment plant producing drinking water from river water (Figure 1). The Northern area is fed by the Méry-sur-Oise plant (treating water from the Oise river), the Eastern area by the Neuilly-sur-Marne plant (treating water from the Marne river) and the Southern area by the Choisy-le-Roi plant (treating water from the Seine river). In each of the three sectors, the maximum residence time of the water at the sampling stations was estimated at between 50 and 60 hours (calculated using SWS hydraulic modelling software by STONER). The treatment in these plants includes: coagulation, flocculation, settling, biological sand or dual media filtration, ozonation, biological granular activated carbon (GAC) filtration and chlorination. In addition, a new treatment train was started up in September 1999 at the Méry-sur-Oise plant; it includes the following stages: clarification, intermediate ozonation, biological dual media filtration, nanofiltration, UV disinfection and final chlorination. Since the building of this new unit, the water feeding the distribution system is a blend of water from the conventional train (usually 30%) and water from the nanofiltration train (70%). Details of the nanofiltration unit were given by Peltier *et al.* (2002).

Sampling campaigns

Between 1990 and 2001, a total of 29 sampling campaigns were performed to investigate the biofilm in the different

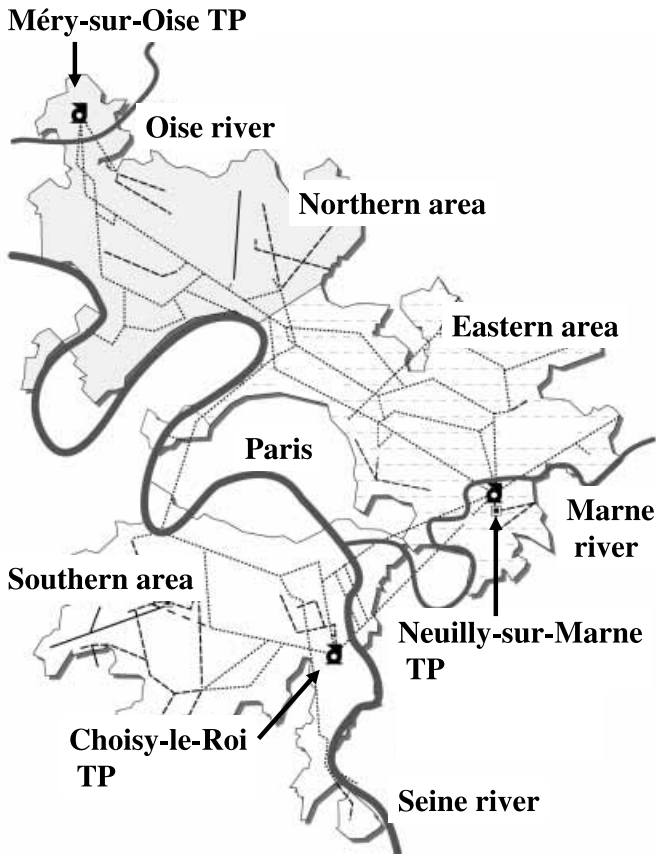

Figure 1 | Map of the three areas of the Parisian suburbs distribution system (with main pipes), the three treatment plants (TP) and the rivers used as raw waters.

sectors of the Parisian suburbs DS (19 campaigns in the Northern area including situations before and after the important treatment modification in 1999; seven in the Eastern area and three in the Southern area). During each sampling campaign, between 7 and 25 sampling stations were investigated from stations close to the treatment plant to stations far from the plant and in dead ends in order to cover a large range of situations in terms of residence time of the water in the DS. At each sampling station, four to eight cast-iron coupons were incubated for 1 month and then collected after colonisation in order to perform measurements on the biofilm (see biofilm sampling section). As the sampling stations were not always exactly the same from one sampling campaign to another, in this paper, we used the average of the data per sampling campaign for results presentation and discussion.

During the long period of the study, in addition to the introduction of the membrane treatment at the Méry-sur-Oise plant (see above), several other modifications were performed in the treatment plants (improvement of clarification stage, introduction of dual media biological filtration instead of biological sand filtration, regeneration or replacement of GAC in the biological filters, etc.) and in the studied DS (installation of rechlorination boosters). So, owing to these modifications and to fluctuations in the raw water quality and plant functioning conditions, our sampling campaigns cover a large range of situations in terms of water quality at the plant outlets and within the DS. The ranges of water quality for the different sampling campaigns, in terms of disinfectant level, dissolved organic carbon (DOC) and biodegradable dissolved organic carbon (BDOC) concentration at the outlet of the plants feeding the three studied DS, are summarised in Table 1.

Biofilm sampling

In order to incubate cast-iron coupons to estimate fixed bacterial biomass, two different systems were used during this study.

1. Chambers, in which direct access to the pipes (mainly 100 mm in diameter) is possible, were built in the Northern part of the Parisian suburbs network (Servais *et al.* 1992*a*). In each of these chambers, there were four sampling devices which permitted the insertion of a coupon of cast iron just at the inner surface of the pipe and in contact with the flowing water (Figure 2). The coupons were subsequently collected after colonisation in the distribution system for biofilm analysis.
2. Colonisation of pipe materials by fixed biomass in the DS was also studied using reactors continuously fed by water from the DS collected at the different sampling points. Each reactor was a polyvinyl chloride (PVC) cylinder (diameter 6 cm, length 50 cm) containing eight circular cast-iron coupons (2.8 cm diameter) fixed in the water flow passing through the reactor (1 to 2 litres per minute). The coupons were collected for biofilm

measurement after colonisation in the distributed water flow.

Cast-iron coupons were used in this study as cast iron is the usual material from which the pipes of the Parisian DS are made; other materials such as PVC are also used especially for small diameter (100 mm) pipes. Before installation for colonisation, the cast-iron coupons were brushed to remove corrosion particles, washed by distilled water and sterilised. With both incubation devices, the coupons were incubated for a period of 1 to 1.5 months. Previous experiments have shown that a period of 1 month was required to reach a stable density of bacterial biomass fixed on coupons incubated in these conditions (Laurent & Servais 1995) (see also 'Results and discussion' section).

Biofilm measurements

Two methods were used in this study to quantify biofilm on cast-iron coupons.

The first method is based on measurement of potential exoproteolytic activity (PEPA) (Somville & Billen 1983), which has been shown to be proportional to bacterial biomass (Billen 1991). In this method, L-leucyl-β-naphthylamide (LL-βN; Sigma-Aldrich, Belgium), a non-fluorescent compound, is added at a saturating concentration to a solution in contact with bacteria and this compound is hydrolysed by bacterial exoenzymes into L-leucyl and β-naphthylamine (βN), a fluorescent compound (Laurent & Servais 1995). Special incubation devices were built to incubate, in the presence of LLβN, only the surface of the cast-iron coupon which has been in contact with the flowing water. Practically, the coupon was incubated in the incubation device for 0.5 to 1 hour with 4 ml of 0.2 μm filtered dechlorinated water (from the same station) and 100 μl of a 34 mM LL-βN solution. At 10 min intervals, a 2 ml subsample was removed, fluorescence was measured at 410 nm under 340 nm excitation using a fluorimeter (Kontron Instruments) and the subsample was replaced in the incubation device. The rate of fluorescence increase, which is a measurement of PEPA, was calculated; a conversion factor of 6.58 μg of bacterial biomass expressed in carbon unit per nmole of βN per minute was used (Laurent & Servais 1995). In these analytical conditions, the detection limit and the reproducibility of this method were estimated at 0.001 μgC cm^{-2}.

A second method was sometimes used in parallel for comparison; it consists of detaching the bacteria from the coupons by sonication and then enumerating the bacteria by epifluorescence microscopy. The conditions of sonication were optimised in order to remove the maximum number of fixed bacterial cells from the cast-iron coupons without cell disruption. The cast-iron coupon (2.8 cm diameter) was placed in a special device allowing the addition of 4 ml of sterile water (0.2 μm filtered dechlorinated water from the DS) in contact with the surface of the cast-iron coupon which has been in contact with the flowing water. Sonication was performed for two 1 minute periods with an interval of 1 minute using a Labsonic (60 W intensity, 1 cm diameter probe). The water was removed from the device and collected; the operation was repeated twice with new sterile water. The water containing the detached bacterial cells was conserved in the presence of formaldehyde (2% final concentration) before bacterial enumeration by epifluorescence microscopy. Usually some metallic particles were removed by this procedure; they greatly interfered with the bacterial enumeration by epifluorescence microscopy when acridine orange staining was used by giving an important fluorescence background (data not shown). This interference was much less with the DAPI staining procedure. In this paper, only data on enumeration of detached cells performed with the DAPI staining procedure as proposed by Porter & Feig (1980) are presented.

Measurements performed on drinking water samples

During each sampling campaign, water samples were collected at the outlet of the treatment plant and at each sampling station where coupons for biofilm analysis were removed. Chlorine, DOC and BDOC were determined as well as the bacterial abundance.

Chlorine concentration

The concentrations of free residual chlorine and total residual chlorine were measured directly at the sampling

Table 1 Range of water quality at the outlet of the treatment plants feeding the three sectors of the Parisian suburbs DS during the biofilm sampling campaigns (*n*=number of sampling campaigns)

Treatment plant	*n*	Free chlorine (mg l^{-1})	DOC (mgC l^{-1})	BDOC (mgC l^{-1})
Méry-sur-Oise (before Sep. 99)	14	0.18–1.05	1.17–3.05	0.20–1.03
Méry-sur-Oise (after Sep. 99)	5	0.15–0.22	0.77–1.09	0.09–0.24
Neuilly-sur-Marne	7	0.29–0.39	1.64–2.32	0.24–0.48
Choisy-le-Roi	3	0.30–0.39	1.65–1.75	0.31–0.35

points using a portable spectrophotometer and the recommended reagents (N,N-diethyl-p-phenylenediamine (DPD) and potassium iodide).

DOC and BDOC

The glassware receiving the water samples for the DOC analysis was muffled at 550°C for 4 hours after cleaning. DOC concentrations were measured using a total organic carbon analyser (Dohrmann DC-180) which uses UV-promoted persulfate oxidation. Dechlorinated samples were previously filtered on carbon-free borosilicate 0.7 μm pore-size filter to remove particulate organic carbon. BDOC concentrations were estimated as the difference of DOC concentrations measured before and after a 30 day incubation at 20°C with indigenous bacteria (1% of 2 μm pore size filtered raw water) (Servais *et al.* 1987, 1989). The accuracy of the method has been estimated at $\pm$ 0.05 mg C l^{-1} (Servais *et al.* 1989).

Bacterial abundance

Bacterial numbers were determined by epifluorescence microscopy after staining cells with a fluorochrome. Acridine orange staining following the procedure of Hobbie *et al.* (1977) was first used; later, DAPI staining following the protocol proposed by Porter & Feig (1980) was used. As we observed that the first procedure slightly underestimated total bacterial numbers compared with the second

Figure 2 | View of a chamber allowing direct access to a 100 mm diameter pipe with two sampling devices which permitted the insertion of a cast-iron coupon just at the inner surface of the pipe.

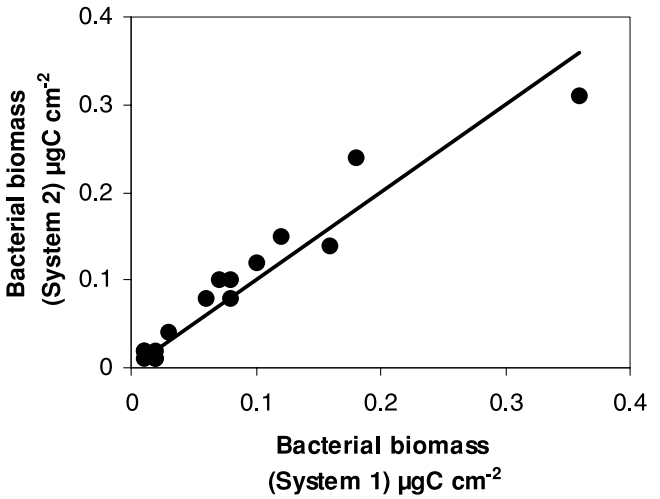

Figure 3 | Fixed bacterial biomass measured by the potential exoproteolytic method on coupons incubated at the same sampling stations either directly at the inner pipe surface (system 1, X axis) or in reactors fed by water from the DS (system 2, Y axis). Correlation straight line passing through the origin: Y (µgC cm^{-2})=1.02 X (µgC cm^{-2}) (r^2=0.92, n=16).

(data not shown), only data obtained with the DAPI staining procedure are presented in this paper.

RESULTS AND DISCUSSION

Impact of coupon incubation procedure on biofilm quantity

On four different sampling stations in the Northern sector of the Parisian suburbs DS the two different systems described above for incubating cast-iron coupons in the DS were compared during four sampling campaigns. That means that, at these stations, one of the four sampling devices which permitted the insertion of a coupon just at the inner surface of the pipe (system 1) was removed and the water coming out of the pipe was used to continuously feed a reactor containing coupons to be colonised (system 2). The coupons in both systems were installed and removed at the same time (after around 1 month of colonisation in the DS) allowing a comparison of bacterial biomass fixed on coupons incubated in both systems. Figure 3 shows that a significant correlation passing through the origin was observed between fixed bacterial biomass incubated in both systems ($r^2 = 0.92$, $n = 16$, $p < 0.01$). The slope of the correlation was very close to 1 showing that fixed bacterial biomass measured on coupons incubated in both systems was similar. As these data demonstrated that the system using reactors continuously fed by water from the DS to incubate coupons allows a correct estimate of the biofilm quantity fixed within the DS, this system is now systematically used as it is less expensive and more practical to use. This system has been successfully used to study the biofilm in various DS (Servais *et al*. 1995; Laurent *et al*. 1999; Niquette *et al*. 2001) and for comparison of colonisation of different pipe materials (Niquette *et al*. 2000).

In order to verify that a 1-month period of incubation of the coupons in the DS was long enough to reach a steady state fixed bacterial biomass, cast-iron coupons were incubated in reactors continuously fed by drinking water from several sampling stations and removed respectively after 1, 2 and 4 months of incubation. No statistically significant difference between the biomass was observed between coupons incubated at one sampling station for different periods of time (data not shown). This confirms that a 1-month incubation can be used for routine estimation of fixed bacterial biomass in the conditions met in the studied DS.

Biofilm measurement with and without bacterial detachment from the support

In this study, the estimate of fixed bacterial biomass was systematically performed by the potential exoproteolytic activity (PEPA) method but, in addition, during ten sampling campaigns, fixed biomass was also estimated by epifluorescence microscopy after bacterial detachment from the cast-iron coupons. Detachment was performed by sonication and bacterial cells were stained by DAPI before microscopic enumeration. Average data per sampling campaign of bacterial abundance expressed in cells per cm^2 were plotted against the average fixed bacterial biomass estimated by the potential exoproteolytic method expressed in µgC cm^{-2} (Figure 4). Figure 4 shows that both estimates were quite well correlated ($r^2 = 0.71$,

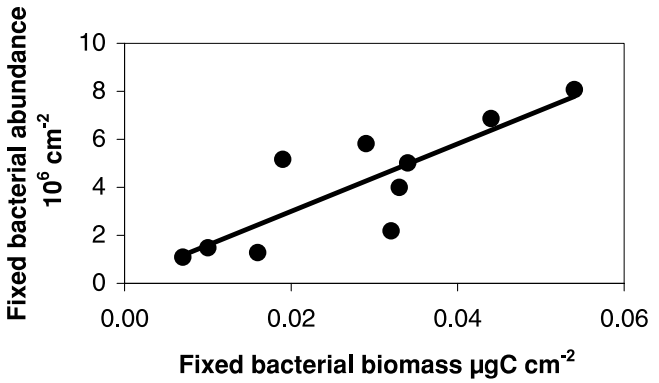

Figure 4 | Fixed bacterial abundance estimated by epifluorescence microscopy after cell detachment by sonication plotted against fixed bacterial biomass estimated by PEPA (data averaged per sampling campaign). Correlation straight line: fixed bacterial abundance (10^6 bacteria cm^{-2})=140.64 fixed bacterial biomass (µgC cm^{-2})+0.19 (r^2=0.71, n=10).

$n = 10$, $p < 0.01$). If we assume that the detachment by sonication was fully efficient (100% removal of attached bacteria), the inverse of the slope of the correlation presented in Figure 4 corresponds to the carbon content per fixed bacterial cell. This calculation gives a carbon content per cell of 7.1 fg which is completely realistic, showing the consistency of both methods.

A carbon content per cell of 7.1 fg is in fact in the lower part of the range of values mentioned in the literature for bacterial cells in aquatic environments (Troussellier *et al*. 1997). As it is known that bacterial cell size and thus the bacterial carbon content are lower in oligotrophic systems than in nutrient rich systems (Billen *et al*. 1990), it is not surprising that the carbon content per cell estimated here was in the lower part of the range of values quoted in the literature as DS can surely be considered as an oligotrophic system for bacteria. An average value of 7 fgC per cell was, for example, recently determined for the bacteria of different sectors of the Atlantic Ocean (Zubkov *et al*. 2000), another oligotrophic environment. Of course, the value of carbon content per cell deduced here depends on the conversion factor used for calculating fixed biomass from PEPA measurements and on the efficiency of sonication to detach cells from pipe coupons.

The comparison performed in this study between microscopic enumeration of bacteria after cell detachment from the support and the PEPA method without cell detachment showed that both methods seem efficient for the estimation of biofilm density fixed on coupons incubated in DS. While sonication followed by microscopic enumeration has been used routinely by several authors to study drinking water DS biofilm (Mathieu *et al*. 1992; Sibille *et al*. 1997), in this study, we chose PEPA for the routine quantification of biofilm. We found this method more rapid (data are obtained after approximately 1 hour's work in the laboratory) and easier to use in the laboratory as it did not require time-consuming bacterial enumeration under epifluorescence microscopy. Some other procedures proposed in the literature for estimating fixed bacterial abundance or biomass were not tested here because they were not found convenient for our purpose. For example, plate count enumeration after bacteria detachment from the support by sonication or scraping has been used in several studies (Donlan & Pipes 1988) but it is now well known that enumeration on a solid medium severely underestimates bacterial abundance and that this underestimation depends on the chlorine concentration (Block 1992). ATP dosage has also been proposed in the literature for biofilm quantification (van der Kooij *et al*. 1995) but as ATP is present in all micro-organisms, it cannot be considered as a good way to estimate fixed bacterial biomass.

Biofilm and suspended bacteria

Bacteria observed in the water phase in a DS can enter into the network with the water feeding the DS or can be due to bacterial development within the DS. It has been previously shown that the production of the bacteria in the water phase estimated by the tritiated thymidine incorporation method (Fuhrman & Azam 1982; Servais *et al*. 1992*b*) was unable to explain the increase of abundance observed with increasing residence time in the distribution system (Servais *et al*. 1992*a*). Thus, it was suggested that the growth mainly occurs in the biofilm and that the pool of suspended bacteria was fed by the detachment of bacterial cells from the biofilm. If this view is correct, a positive relationship between the biofilm density and the suspended bacteria abundance should be observed. In a

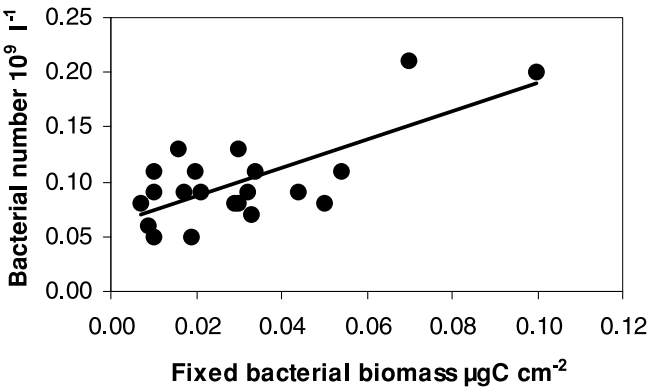

Figure 5 | Average free bacterial abundance plotted against average fixed bacterial abundance for the different sampling campaigns in the Parisian suburbs networks. Correlation straight line: suspended bacterial abundance (10^9 l^{-1})=1.29 fixed bacterial biomass (µgC cm^{-2})+0.06 (r^2=0.52, n=21).

previous study comparing the bacterial dynamics in various French distribution networks, Servais *et al.* (1995) showed a linear relationship between the abundance of suspended and fixed bacteria in DS. In the same way, with the present set of data, a significant correlation ($r^2 = 0.52$, $n = 21$, $p < 0.05$) was observed between the average bacterial abundance in the water phase and the biofilm density estimated by the PEPA method (Figure 5).

Although the relationship was statistically significant, an important scatter was, however, observed. This is probably due to the fact that the detachment of cells from the biofilm in the DS is, in addition to the slow and constant release mechanism linked to the growth of fixed bacteria, a transient process occurring when shear stress increases owing to modifications in the hydraulic conditions. If the increase of suspended bacteria within the DS is due to a feeding of this pool by cell detachment from the biofilm, logically the Y intercept of the relationship presented in Figure 5 should represent the suspended bacteria entering the DS, that is, the abundance of bacteria in the water leaving the treatment plant. The value of the Y intercept in Figure 5, around 0.06×10^9 cells l^{-1}, is just in the middle of the range (0.03×10^9 to 0.1×10^9 cells l^{-1}) of the bacterial abundance in the water feeding the DS for the sampling campaigns considered in Figure 5.

The relationship shown in Figure 5 indicated that when an average biofilm of 0.05 µgC cm^{-2} corresponding to a fixed bacterial abundance of 7.2×10^6 cells per cm^{-2} (calculated by the correlation observed in Figure 4) is measured, the abundance of suspended bacteria should be on average equal to 120×10^6 cells per litre. From these values, it is easy to calculate that, in a 100 mm diameter pipe where the ratio between volume and surface area is 25 l m^{-2}, the ratio of fixed to free bacteria is around 25, meaning that fixed bacteria represent a 25 times more important biomass than the bacteria in the water phase. As quite similar growth rates were previously measured in DS for suspended and fixed bacteria by thymidine incorporation (Servais *et al.* 1992*a*), it means that bacterial production in the water phase is negligible with regard to the production occurring in the biofilm fixed on the pipe surface. The data presented here completely confirm the view of feeding of the bacteria to the water phase by cells growing in the biofilm.

These data also demonstrate that to maintain a good microbiological water quality at the tap of the consumers it is of major importance to limit the biofilm present on the DS inner pipe walls which serves as a source of suspended bacteria in the distributed water.

Factors controlling biofilm in DS

In order to limit the formation of the biofilm in DS, most of the water utilities used chlorination of treated water and tried to maintain a chlorine residual everywhere in the DS. In the present study, the impact of chlorine on biofilm was first investigated by comparing the biofilm density on coupons incubated in water with or without a significant chlorine residual. For this, cast-iron coupons were incubated in reactors fed at the Méry-sur-Oise treatment plant, on the one hand, by biologically GAC filtered water and, on the other hand, by water feeding the DS (chlorinated water). Both waters contain roughly the same amount of BDOC as chlorination did not significantly affect the BDOC content of the water (data not shown); the GAC filtered water did not contain any disinfectant residual while the chlorine content of the treated water was, on average during the period of the study, equal to 0.4 mg l^{-1}. Average fixed bacterial biomass measured on several cast-iron coupons incubated for 1 month in both types of

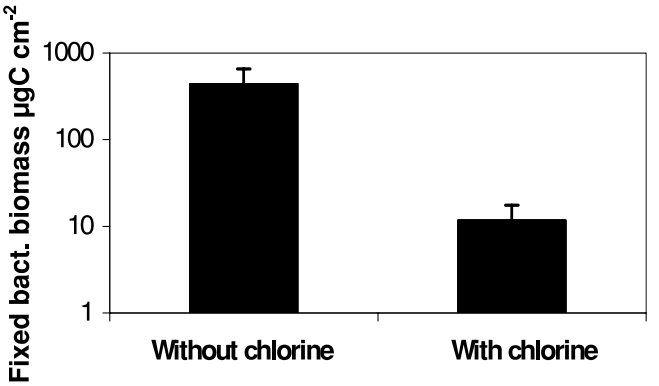

Figure 6 | Fixed bacterial biomass estimated by the PEPA method on cast-iron coupons incubated in treated water from the Méry-sur-Oise treatment plant (plotted in logarithmic scale) in the absence and in the presence of chlorine (average concentration during the coupon incubation period: 0.4 $mgCl_2\ l^{-1}$).

water is presented in Figure 6. The biomass on the coupons incubated in biologically GAC filtered water was roughly 40 times greater than that estimated on the coupons incubated in chlorinated water. In this experiment, the impact of chlorine on the biofilm quantity is drastic: a constant 0.4 mg l^{-1} residual reduces the biofilm by a factor of 40.

The impact of chlorine residual can also be clearly observed in Figure 7 in which the logarithm of average fixed bacterial biomass per sampling campaign was plotted against the average chlorine residual within the DS for the corresponding campaign. In order to avoid too large an interference of the BDOC content of the water (see below) on biofilm quantity, only data concerning biologically treated water at the plant were considered in Figure 7. This figure confirms the strong impact of chlorine, showing, for example, that an increase of free chlorine from an average value of 0.05 to 0.2 mg l^{-1} allows a reduction of the biofilm by an order of magnitude. This data is completely in concordance with previous data obtained on pilot DS. For example, Mathieu *et al.* (1992) also showed a linear correlation between the logarithm of the abundance of attached cells (estimated by epifluorescence microscopy after detachment by sonication) and the chlorine residual in a pipe loop system used as a model DS. The relationship presented by these authors indicated a one log decrease of biofilm for a chlorine increase from 0.05 to 0.3 mg l^{-1}.

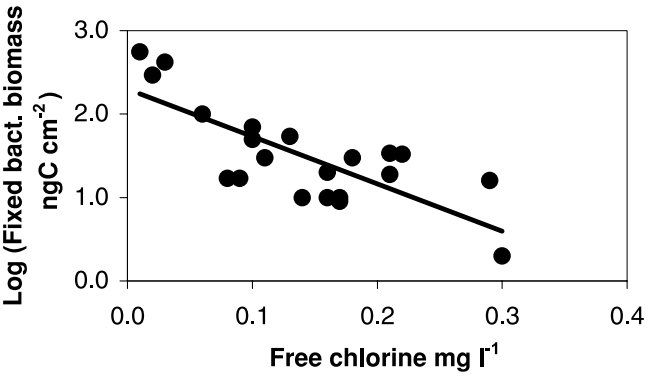

Figure 7 | Logarithm of the average fixed bacterial biomass (expressed in ngC cm^{-2}) measured on cast-irons coupons by the PEPA method plotted against the average chlorine residuals for different sampling campaigns in the Parisian suburbs networks (situations with nanofiltration treatment at the plant were not considered for this figure). Correlation straight line: log (fixed bacterial biomass µgC cm^{-2}) = −5.67 free chlorine (mg l^{-1}) + 2.30 (r^2 = 0.59; n = 21).

However, as the chlorine residual rapidly decreased in the DS with increasing residence time owing to reactions with dissolved organic matter and pipes walls, it is not possible to maintain a chlorine residual throughout each DS without increasing chlorine application to high levels. Applying high levels of chlorine is not always a realistic solution to control biofilm, as chlorination of water leads, by reaction with dissolved organic carbon, to the formation of undesirable disinfection by-products such as trihalomethanes (THM) for which permissible levels are continuously being reduced by increasingly strict standards. Thus, an alternative solution proposed in the literature to limit bacterial development in DS is to decrease the supply of the substrate used by bacteria to grow, that is, the biodegradable dissolved organic carbon (BDOC).

Several studies (LeChevallier *et al.* 1991; van der Kooij 1992; Servais *et al.* 1992*a*, 1995; Volk & LeChevallier 1999) have shown that reducing organic matter in treated drinking water is an efficient way to avoid bacterial regrowth in DS. Some of these studies have proposed thresholds in terms of BDOC in the inlet water for biological stability even in the absence of disinfectant residual. For example, Servais *et al.* (1995) have shown that for BDOC values around or lower than 0.15 mgC l^{-1} the water can be considered as biologically stable on the basis of a comparison of the BDOC and bacterial dynamics in several French

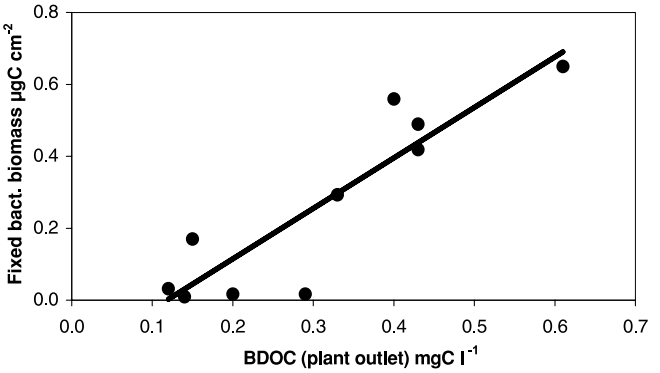

Figure 8 | Average fixed bacterial biomass (expressed in µgC cm^{-2}) measured on cast-iron coupons plotted against the BDOC at the plant outlet for different sampling campaigns in the Parisian suburbs networks (only situations with an average free chlorine residual in the DS<0.1 $mgCl_2$ l^{-1} were considered for this figure). Correlation straight line: fixed bacterial biomass (µgC cm^{-2})=1.40 BDOC (mgC l^{-1})−0.17 (r^2=0.81; n=10).

DS. Reducing the BDOC in the treated water provides, in addition, two other advantages: it reduces the formation of organochlorine compounds during chlorination and it decreases chlorine consumption rate in the network and thus improves the stability of the disinfectant residual during distribution.

In the present study, in order to estimate the impact of the BDOC content of the treated water on biofilm in DS, we have plotted the average fixed bacterial biomass for different sampling campaigns performed in the Parisian suburbs DS against the BDOC concentration of the treated water. In order to avoid the interference of the impact of chlorine on biofilm, which is known to be very important (see above), only the sampling campaigns for which the average free chlorine residual within the DS was lower than 0.1 mg l^{-1} were considered. Figure 8 shows that reducing BDOC in the inlet water reduces the quantity of biofilm. It also demonstrates that, even with a very low chlorine residual (<0.1 mg l^{-1}), the fixed bacterial biomass is very low when the BDOC concentration is low (<0.2 mg l^{-1}). Thus using nanofiltration treatment to reduce the BDOC in the treated water, as is currently done in one Parisian suburbs treatment plant (Méry-sur-Oise plant), seems a very efficient way to limit biofilm and to increase bacteriological water quality at the same time as limiting chlorine application and thus reducing THM formation (Peltier *et al.* 2002).

The results gained during this study clearly demonstrated on full scale DS data that chlorine concentration and BDOC of the treated water are the two major controlling factors for the fixed bacterial biomass. As shown by several studies (Pedersen 1990; Percival *et al.* 1998; Niquette *et al.* 2000), another important factor influencing the biofilm quantity is the pipe material; however, this factor was not taken into account in the present study as all our measurements were performed on cast-iron coupons.

CONCLUSION

From a methodological point of view, this study presents data showing that quite a simple incubation system can be used in DS to colonise coupons in order to estimate biofilm. The data showed that a similar biofilm quantity was found on cast-iron coupons incubated in the simple reactor proposed here to that found on similar coupons incubated directly on the inner pipe surface in the DS. The reactors for coupon incubation are inexpensive and can be connected everywhere in a DS in order to estimate the level of fixed bacterial biomass and its spatial distribution in a given DS. Controlling biofilm quantity seems important for distributed water microbiological quality because most of the bacteria present in a DS are within the biofilm and because bacterial growth within DS occurs mainly in the biofilm. The large set of data gained on biofilm within the Parisian suburbs DS used in this paper allows us to demonstrate, using full scale data, the major role of the chlorine residual and BDOC as controlling factors for the biofilm in DS.

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REFERENCES

Appenzeller, B. M. R., Batte, M., Mathieu, L., Block, J. C., Lahoussine, V., Cavard, J. & Gatel, D. 2001 Effect of adding phosphate to drinking water on bacterial growth in slightly and highly corroded pipes. *Wat. Res*. **35**, 1100–1105.

Billen, G. 1991 Protein degradation in aquatic environments. In *Microbial Enzyme in Aquatic Environments* (ed. R. Chrost), pp. 348–389. Springer Verlag, Berlin.

Billen, G., Servais, P. & Becquevort, S. 1990 Dynamics of bacterioplankton in oligotrophic & eutrophic aquatic environments: bottom-up or top-down control? *Hydrobiologia* **207**, 37–42.

Block, J. C. 1992 Biofilms in drinking water distribution systems. In *Biofilms: Science and Technology* (ed. T. R. Boh, L. Melo, M. Fletcher & B. Capdeville), pp. 469–485. Kluwer Publishers, Dordrecht, The Netherlands.

Butterfield, P. W., Bargmeyer, A. M., Camper, A. & Biederman, J. A. 2002 Modified enzyme activity assay to determine biofilm biomass. *J. Microb. Meth*. **50**, 23–31.

Donlan, R. P. & Pipes, W. O. 1988 Selected drinking water characteristics and attached microbial populations density. *J. Am. Wat. Wks Assoc*. **80**, 70–76.

Emde, K. M. E., Smith, D. W. & Facey, R. 1992 Initial investigation of microbially influenced corrosion (MIC) in low-temperature water distribution system. *Wat. Res*. **26**, 169–175.

Fuhrman, J. A. & Azam, F. 1982 Thymidine incorporation as a measure of heterotrophic bacterioplankton production in marine surface waters: Evaluation and field results. *Mar. Biol.* **66**, 109–120.

Haudidier, K., Paquin, J. L., Français, T., Hartemann, P., Grapin, G., Colin, F., Jourdain, M. J., Block, J. C., Cheron, J., Pascal, O., Levi, Y. & Miazga, J. 1988 Biofilm growth in a drinking water network: a preliminary industrial pilot-plant experiment. *Wat. Sci. Technol*. **20**, 109–115.

Hobbie, J. E., Daley, R. J. & Jaspers, S. 1977 Use of Nuclepore filters for counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol*. **33**, 1225–1228.

Holt, D. M., Woodward, T., Rachwal, T., Delanoue, A. & Colbourne, J. S. 1994 A pipe based experimental system to study factors influencing bacterial growth in London's water mains. *Proceedings Water Quality Technology Conference, San Francisco 1994*, pp. 399–414.

Laurent, P. & Servais, P. 1995 Fixed bacterial biomass estimated by potential exoproteolytic activity. *Can. J. Microbiol*. **41**, 743–752.

Laurent, P., Servais, P., Gatel, D., Randon, G., Bonne, P. & Cavard, J. 1999 Microbiological quality before and after nanofiltration. *J. Am. Wat. Wks Assoc.* **91**(10), 62–72.

LeChevallier, M. W. 1990 Coliform regrowth in drinking water: a review. *J. Am. Wat. Wks Assoc*. **82**(11), 74–86.

LeChevallier, M. W., Babcock, T. M. & Lee, R. G. 1987 Examination and characterization of distribution systems biofilms. *Appl. Environ. Microbiol*. **53**, 2714–2724.

LeChevallier, M. W., Shaw, N. & Smith, D. B. 1991 Bacterial nutrients in drinking water. *Appl. Environ. Microbiol*. **57**, 857–862.

Lévy, R. V., Hart, F. L. & Cheetham, R. D. 1986 Occurrence and public health significance of invertebrate in drinking water systems. *J. Am. Wat. Wks Assoc*. **78**(9), 105–110.

Mallevialle, J. & Suffet, I. H. 1987 *Identification and Treatment of Tastes and Odors in Drinking Water*, AWWA Research Foundation and Lyonnaise des Eaux (eds). AWWA Publishers, Denver, Colorado.

Mathieu, L., Paquin, J. L., Block, J. C., Randon, G., Maillard, J. & Reasoner, D. 1992 Paramètres gouvernant la prolifération bactérienne dans les réseaux de distribution d'eau. *Sciences de l'Eau* **5**, 91–112.

Niquette, P., Servais, P. & Savoir, R. 2000 Impacts of pipe materials on densities of fixed bacterial biomass in a drinking water distribution system. *Wat. Res.* **34**, 1952–1956.

Niquette, P., Servais, P. & Savoir, R. 2001 Bacterial dynamics in the drinking water distribution system of Brussels. *Wat. Res*. **35**, 96–103.

Pedersen, K. 1990 Biofilm development on stainless steel and PVC surfaces in drinking water. *Wat. Res.* **24**(2), 239–243.

Peltier, S., Benezet, M., Gatel, D. Cavard, J. & Servais, P. 2002 Improvement of water quality in distribution system by nanofiltration treatment. *J. Wat. Suppl.: Res. & Technol.-AQUA* **51**, 253–262.

Percival, S. L., Knapp, J. S., Edyvean, R. & Wales, D. S. 1998 Biofilm development on stainless steel in mains water. *Wat. Res.* **32**(1), 243–253.

Porter, K. G. & Feig, Y. S. 1980 Use of DAPI for identifying and counting aquatic microflore. *Limnol. Oceanogr*. **25**, 943–948.

Rigway, H. F. & Olson, B. H. 1981 Scanning electron microscope evidence for bacterial colonization of bacteria from potable water. *Appl. Environ. Microbiol*. **49**, 1–7.

Servais, P., Billen, G. & Hascoët, M. C. 1987 Determination of the biodegradable fraction of dissolved organic matter in waters. *Wat. Res*. **21**, 445–450.

Servais, P., Anzil, A. & Ventresque, C. 1989 Simple method for the determination of biodegradable dissolved organic carbon in water. *Appl. Environ. Microbiol*. **55**, 2732–2734.

Servais, P., Billen, G., Laurent, P., Levi, Y. & Randon, G. 1992a Studies of BDOC and bacterial dynamics in the drinking water distribution system of the Northern Parisian suburbs. *Sciences de l'Eau* **5**, 69–89.

Servais, P., Laurent, P. & Randon, G. 1992b Mesure de la biomasse et de l'activité bactérienne dans l'eau de distribution. *Sciences de l'Eau* **5**, 473–488.

Servais, P., Laurent, P. & Randon, G. 1995 Comparison of the bacterial dynamics in various French distribution systems. *J. Wat. Suppl.: Res. & Technol.-AQUA* **44**(1), 10–17.

Sibille, I., Mathieu, L., Paquin, J. L., Gatel, G. & Block, J. C. 1997 Micro-organisms characteristics of a distribution system fed with nanofiltered drinking water. *Wat. Res.* **31**, 2318–2326.

Somville, M. & Billen, G. 1983 A method for determination exoproteolytic activity in natural waters. *Limnol. Oceanogr.* **28**, 190–193.

Troussellier, M., Bouvy, M., Courties, C. & Dupuy, C. 1997 Variation of carbon content among bacterial species under starvation condition. *Aquat. Microbiol. Ecol.* **13**, 113–119.

Van der Kooij, D. 1992 Assimilable organic carbon as an indicator of bacterial regrowth. *J. Am. Wat. Wks Assoc.* **84**(2), 57–65.

Van der Kooij, D., Veenendaal, H. R., Baarst-Lorist, C., Van der Klift, D. W. & Drost, Y. C. 1995 Biofilm formation on surface of glass and teflon exposed to treated water. *Wat. Res.* **29**, 1655–1662.

Van der Wende, R., Characklis, W. G. & Smith, D. B. 1989 Biofilms and bacterial drinking water quality. *Wat. Res.* **23**, 1313–1322.

Volk, C. J. & LeChevallier, M. W. 1999 Impacts of the reduction of nutrient levels on bacterial water quality in distribution systems. *Appl. Environ. Microbiol.* **65**(11), 4957–4966.

Zubkov, M. V., Sleight, M. A., Burkill, P. H. & Leakey, R. J. G. 2000 Bacterial growth and grazing loss in contrasting areas of North and South Atlantic. *J. Plankton Res.* **22**, 685–711.

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