This article was published in Water Research 62, 63-87, 2014

http://dx.doi.org/10.1016/j.watres.2014.05.039

An Overview on the Reactors to Study Drinking Water Biofilms

Gomes, I. B.[a], Simões, M.[a] and Simões, L. C.[a,b*]

[a] LEPABE, Department of Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, s/n, 4200-465 Porto, Portugal

[b] CEB- Centre of Biological Engineering, University of Minho, Campus de Gualtar 4710-057 Braga, Portugal.

*Corresponding author: Lúcia C. Simões, Centro de Engenharia Biológica, Universidade do Minho, Tel: 00351 253604404. Fax: 00351 253678986. E-mail: luciachaves@deb.uminho.pt

ABSTRACT

The development of biofilms in drinking water distribution systems (DWDS) can cause pipe degradation, changes in the water organoleptic properties but the main problem is related to the public health. Biofilms are the main responsible for the microbial presence in drinking water (DW) and can be reservoirs for pathogens. Therefore, the understanding of the mechanisms underlying biofilm formation and behavior is of utmost importance in order to create effective control strategies. As the study of biofilms in real DWDS is difficult, several devices have been developed. These devices allow biofilm formation under controlled conditions of physical (flow velocity, shear stress, temperature, type of pipe material, etc), chemical (type and amount of nutrients, type of disinfectant and residuals, organic and inorganic particles, ions, etc) and biological (composition of microbial community – type of microorganism and characteristics) parameters, ensuring that the operational conditions are similar as possible to the DWDS conditions in order to achieve results that can be applied to the real scenarios. The devices used in DW biofilm studies can be divided essentially in two groups, those usually applied *in situ* and the bench top laboratorial reactors. The selection of a device should be obviously in accordance with the aim of the study and its advantages and limitations should be evaluated to obtain reproducible results that can be transposed into the reality of the DWDS. The aim of this review is to provide an overview on the main reactors used in DW biofilm studies, describing their characteristics and applications, taking into account their main advantages and limitations.

Keywords: Biofilm control; Biofilm monitoring; Drinking water; Reactor

1. Introduction

There is a global concern that all the world population should have access to safe drinking water (DW). Even in the 21st century, there are many people without access to appropriate

48 water, in quantity and/or quality, for the basic needs (WHO, 2011). The existence of DW
49 distribution systems (DWDS) allows the management and supply of water for more people.
50 However, there are several problems that can occur in a DWDS. From a microbiological
51 perspective, the main problems reported in DWDS are the biocorrosion, biofilm formation,
52 nitrification and also the occurrence and persistence of pathogenic organisms (Beech and
53 Sunner, 2004; Camper, 2004; Emtiazi et al., 2004; Simões and Simões, 2013; Teng et al.,
54 2008). Biofilms are considered to be the main source of microorganisms in DWDS that are
55 fed with treated water (Berry et al., 2006; Yu et al., 2010). Biofilms are a set of
56 microorganisms attached to a surface through exopolymers they produce, also known as
57 extracellular polymeric substances (EPS). These are mainly proteins and polysaccharides that
58 are involved in microbial protection from stress conditions (Fang et al., 2010). The main
59 microorganisms that are commonly detected in water are heterotrophic bacteria, particularly
60 α-,β- and γ-proteobacteria (Berry et al., 2006), mycobacteria, some filamentous fungi, virus
61 and helminths (Abe et al., 2011). The existence of inorganic matter, like corrosion products,
62 clays and sand, can be responsible for changes in biofilm structure, increasing its mechanical
63 cohesion (Melo and Bott, 1997). Biofilms occur usually on surfaces which are in contact with
64 water. So, biofilm formation is common in DWDS. Wingender and Flemming (2004) stated
65 that 95% of water microorganisms are present in DWDS inside biofilms while only 5% are
66 floating in the bulk phase.
67 Although biofilms are the main form of microbial organization in nature, the formation of
68 these structures in DWDS depends of several biotic and abiotic factors, namely
69 environmental factors (temperature and pH), concentration of residual disinfectants, nature
70 and concentration of nutrients, hydrodynamic conditions (flow rate, design of network and
71 presence of dead ends), type of pipe materials and their conservation state, type and diversity

of microorganisms present and sediment accumulation (Deines et al., 2010; Jang et al., 2011; Simões and Simões, 2013; Yu et al., 2010). The biofilm formation process occurs in several steps (Fig. 1) (O'Toole et al., 2000). The preconditioning of the pipe surface by organic and inorganic macromolecules facilitates the bacterial adhesion process. Thereafter, cells can adsorb to the surface reversibly or irreversibly (a). After adhesion, a stage of active biofilm growth occurs by cell replication, EPS production, release of quorum-sensing (QS) molecules and exchange of substances between the biofilm and the bulk (b and c). The biofilm dispersion and formation/colonization in other clean areas can take place after biofilm detachment from pipes walls, as depicted in Fig. 1 (d, e and f) (Codony et al., 2005). The amount of a biofilm in a given system, after a certain period of time, depends on a dynamic biofilm formation process, which has been defined as the balance between bacterial attachment from the planktonic phase, bacterial growth within the biofilm and dynamic detachment from the surface (Stoodley et al., 1999). When the balance is null, the biofilm is said to have reached a steady-state. The final amount of biofilm in that state, which can be assessed by cell counts or biomass determination, is directly related to its formation potential in the system (Van der Kooij, 1999). Hydrodynamics have an utmost role in biofilm development and in determining its stability (Bott, 1993). The flow rate affects biofilm development by interfering with several phenomena, namely: nutrients transport, bacterial adhesion, biofilm growth and detachment (Characklis and Marshall, 1990). When the flow velocity is low there is a high resistance to mass transfer (nutrients, oxygen, etc.) from the bulk fluid to the microorganisms embedded in biofilms, impairing sessile cell growth. On the other hand, high flow velocity causes high turbulence of the fluid bulk. It means that the mass transfer phenomena are enhanced, improving also the biofilm growth. However, high velocity also causes high shear forces that can be responsible for higher biofilm erosion and

detachment; accordingly it may cause a decrease of biofilm mass on surfaces. Therefore, apart from others factors, studies on the effects of hydrodynamic conditions are also very important to understand biofilm formation in DWDS. However, the hydrodynamic conditions (flow rate, velocity, residence time, shear stress) are dependent of the geometry of each biofilm reactor. The dimensionless Reynolds number (Re), in fluid mechanics, is defined as the ratio of inertial forces to viscous forces and is used to describe the flow conditions of a fluid (laminar, transition and turbulent flow). Its calculation is dependent of the reactor flow geometry. Also, the definition of laminar and turbulent flow regimes varies according to the system used. The Re number for the flow in a pipe or tube can be defined by Eq. (1) and (2) where D_H is the hydraulic diameter of the pipe (m), ρ is the fluid density (kg.m^{-3}), v is the flow velocity (m.s^{-1}), μ is the dynamic viscosity of fluid (N.s.m^{-2}), A is the pipe cross sectional area (m^2) and P is the wetted perimeter (m). The wetted perimeter for a pipe is the perimeter of the pipe wall that is in contact with the water flow.

$$Re_{pipe} = \frac{\rho v D_H}{\mu} \quad (1)$$

$$D_H = \frac{4A}{P} \quad (2)$$

In cylindrical pipes, Re < 2300, 2300 <Re < 4000, Re > 4000, correspond to laminar, transition and turbulent flow conditions, respectively. The Re number for a stirred tank is defined by the Eq. (3) where N is the rotational velocity and D is the diameter of agitator. For an agitation situation the laminar flow is considered when Re < 10 and turbulent flow for Re > 10^4 (Pérez et al., 2006).

$$Re_{stirred\ tank} = \frac{ND^2\rho}{\mu} \quad (3)$$

One of the major obstacles to study biofilms within DWDS is how to choose a suitable experimental system that mimics the conditions found in real pipe networks. A number of

devices have been described in literature for studying biofilms in DWDS. Therefore, the aim of this review is to provide an overview on old and well described and reviewed biofilm reactors as well as on new or more recently developed reactors that not have been reviewed together yet. The diverse devices are described as well as aspects on their limitations and advantages. Also, a brief description on the main applications of reactors in DW biofilm studies and the quantification methods used for DW biofilm characterization is provided. Nevertheless, the complexity of the DWDS microenvironment and even the use of different methodologies and biofilm reactors have led in some cases to ambiguous or not easily comparable results. Most studies assessed only one variable at a time, and apart from notable exceptions, few attempts have been made so far to study their inter-relationships and compare the relative importance of these different factors.

2. Bench top laboratorial devices

Several devices were developed to study biofilms autonomously from DWDS. These devices try to mimic the DWDS behavior, allowing testing different conditions and can be fed with tap water or with appropriate medium or enriched water. In fact, these devices are DWDS models used to achieve a diversity of goals. However, they were used mostly in laboratorial experiments.

2.1. Annular Reactor

The annular reactor can operate as an open/continuous system and has been used for several decades for the development of biofilms under turbulent flowing environments (Morin and Camper, 1997; Volk and LeChevallier, 1999). It is a simple reactor that mimics the hydrodynamic behavior that biofilms are subjected in real DWDS (Batté et al., 2003a; Keinänen-Toivola et al., 2006). This reactor, also known as Rototorque®, is constituted by

two cylinders, one static external cylinder and other rotating internal cylinder whose speed is controlled by a motor (Chandy and Angles, 2001; Fang et al., 2010; Hosni et al., 2011; Morin and Camper, 1997; Zhou et al., 2009). Usually, the inner cylinder supports some coupons used to sample the biofilm (Fig. 2). The rotation of the inner cylinder is controlled in order to define the desired shear stress. The relationship between shear forces, the cylinder diameter and the rotational speed is provided in Table 1. However, the equations are a gross simplification of the annular reactor shear stress determination, since its calculation for this particular system is quite complex.

The shear stress usually described as characteristic of DWDS pipes is 0.25 $N.m^{-2}$ that is equivalent to 0.3 $m.s^{-1}$ in a 100 mm diameter pipe; these conditions are often reproduced in the annular reactor (Butterfield et al., 2002; Fang et al., 2010; Gagnon et al., 2004, 2005; Jang et al., 2011, 2012, Morin and Camper, 1997; Murphy et al., 2008; Pintar and Slawson, 2003; Szabo et al., 2007). One value of flow velocity that is also often used is 0.6 $m.s^{-1}$ (Batté et al., 2003a, 2003b; Sharp et al., 2001). Rand et al. (2007) tested a shear force of 0.68 $N.m^{-2}$ to assess the efficiency of chlorine dioxide or chlorine coupled with UV treatment on DW biofilm control. The data shown that, the combination of chlorine dioxide/UV was the most effective strategy against both suspended and attached bacteria. Altman et al. (2009) studied the integration and retention of planktonic pathogen *Bacillus cereus* in a *Pseudomonas fluorescens* biofilm under a range of different hydraulic conditions (from 0.15 to 1.5 $N.m^{-2}$ or from 50 to 300 rpm). The authors found that the amount of pathogens detected in the biofilms was higher in the mid-shear range.

This reactor also has been used to study the influence of temperature on biofilm development. Some annular reactors have a jacket allowing working at the desired temperature. Pintar and Slawson (2003) tested different temperatures (6, 12 and 22 ºC) and different concentrations

of disinfectant residual (chloramination) being the reactor fed with tap water and working at constant rotation speed (50 rpm) providing a shear stress of 0.25 $N.m^{-2}$. The results clearly indicate that biofilm development occurs at all examined temperatures, as well as at the selected monochloramine residuals. However, the maintenance of a disinfectant residual had more biofilm inhibitory effects than that of the low temperature. Ndiongue et al. (2005) also studied the effect of temperature (6, 12 and 18 ºC) and biodegradable organic matter on biofilm control by chlorine at 92 rpm. Overall, the results shown that both temperature and nutrients levels are important factors that must be considered when using free chlorine residual to control DW biofilms.

With the aim to perform different studies and save resources, variations of the conventional annular reactor were developed. An example is the conical annular reactor. A standard annular reactors provides a constant wall shear stress distribution on surfaces, while a conical annular reactor generates a non-uniform distribution of this hydrodynamic strength. Rochex et al. (2008) used a conical annular reactor (CCTR - Conical Couette-Taylor reactor) to develop biofilms at varying shear stresses (0.055 to 0.27 Pa from bottom to top of the reactor) with only one device and provided a useful model for studying the effect of hydrodynamics on biofilms. These authors also evaluated the effects of shear stress on the bacterial biofilm community composition. The results shown that, high shear stresses decreased biofilm diversity and slowed down its maturation, maintaining the characteristics of young biofilms. The use of annular reactors to study DW biofilm development and control can be advantageous, mainly if the objective of the work is to study the material influence (allows the study of different materials at the same time) or the effect of hydrodynamics. This reactor also allows to take a considerable number of samples for each assay and has an easy sampling process. The control of shear stress and linear velocity is also simple since it is determined

by the rotational velocity of the internal cylinder and thereafter it is independent from the water flow rate fed to the reactor. So, the residence time and loading rate can be controlled independently. Nevertheless, as referred above, the description of hydrodynamic equations in annular reactors is complex once the flow on cylindrical surface is not well defined due to the presence of Taylor vortices (Childs, 2011). Therefore, the shear stress is not uniform in all surfaces available for biofilm formation.

2.2. Concentric cylinder reactor

The concentric cylinder reactor (CCR) was firstly described and used to study biofilm formation in the dairy industry (Willcock et al., 2000). This reactor allows the simultaneous generation of different shear rates on the same inoculating population (Willcock et al., 2000), but not with the same water phase since the four chambers are fed independently (Fig. 3). Latter, this reactor was used to study DW biofilms by Rickard et al. (2004), who described the effects of different shear forces on DW biofilms formation and its impacts on the microbial community diversity. This reactor is composed by four rotating cylinder pipes and four stationary cylinder chambers (Fig. 3). The chambers can be feed with tap water and the volume inside the chambers is constant and controlled with the help of external pumps, being the feeding ports different from the outlet and sampling ports. The shear stress is controlled with the rotational velocity and radius of the cylinders. Rickard et al. (2004) used this reactor with cylinders whose diameter was 101, 77, 50 and 26 mm that corresponds to fluid velocity of 0.26, 0.19, 0.16 and 0.12 m/s and shear rates of 305, 198, 122 and 65 s^{-1}, respectively. The rotational speed of cylinders was kept constant during all the work (43 rpm), while the shear force varied with the radius of the rotating surface. The fluid velocity profiles were determined on the basis of computational fluid dynamics and from each fluid velocity profile,

shear rates were calculated. The results demonstrated that shear rates affect biofilm diversity as well as the relative proportions of aggregating bacteria. An inverse relationship between shear rate and biofilm diversity was found and the proportions of aggregating bacteria in biofilms also change in relation to shear rates. The authors suggested that it is likely that such cell-cell interactions aid in the integration of bacteria in flowing environments.

This reactor is interesting to study simultaneously the effects of different shear stresses on DW biofilm, allowing to mimic what happens with DWDS since along the distribution system there are variations on water flow velocity. However, it only allows studying one material for each assay and the sampling process is not very easy, since it is necessary the harvesting of biofilm samples from the cylinder surface.

2.3. Flow cell system

The flow cell system consists in a duct segment where removable coupons are inserted in the inner wall, whose allows the biofilm sampling over time. But, this system may present different configurations. The flow cell reactor can be a semicircular duct with some coupons (only the upper face contacts with water) located on the flat wall and the flow pass-through the duct from the bottom to top (Fig. 4). Another flow cell configuration can be a parallel plate flow cell reactor, which consists in a rectangular flow channel with small removable coupons inside, to monitor biofilm formation (Huang et al. 1992). Usually, the flow cell reactor is provided by a feed/fresh water reservoir and the temperature can be controlled externally. The flow is recirculated and the sampling process do not stop the flow because outlet ports are located in the curved wall between two removal coupons, allowing the deviation of flow (Manuel et al., 2007; Simões et al., 2006, 2012). Therefore, this system also allows mimicking the DWDS conditions, since it is a versatile system that allows

239 periodical sampling, without stopping the flow, and the flow velocity can be controlled by
240 an external pump. However, the boundary of sampling coupons can change the water flow,
241 which can affect biofilm development. Flow cell reactors can be used to monitor biofilm
242 development and behavior face to different control treatments and also to test the influence
243 of different materials and hydrodynamic conditions on biofilm formation. This reactor also
244 can be used as an *in situ* device, acting as a by-pass in DWDS. As example, Simões et al.
245 (2006) used the flow cell reactor to monitor biofilms exposed to different operational
246 conditions. The flow cell reactor was fed with tap water without chlorine, previously removed
247 with activated carbon filters. The influence of diverse conditions on biofilm formation were
248 studied, namely the turbulent (4000 $L.h^{-1}$, Re = 11000) and laminar (73 $L.h^{-1}$, Re = 2000)
249 flow, the presence and absence of nutrients (C, P and N) and the type of surface materials,
250 stainless steel (SS) and polyvinyl chloride (PVC). This study allowed to conclude that from
251 the most relevant to the least relevant factor, the biofilms increased due to the addition of
252 nutrients to water; the use of turbulent instead of laminar hydrodynamic flow; and the use of
253 PVC instead of SS as the support material.
254 Manuel et al. (2007) studied the influence of different materials on biofilm development and
255 the effects of the flow and non-flow regimes on the growth of both attached and suspended
256 bacteria using a flow cell reactor. The reactor was fed with tap water at 15.1 $mL.d^{-1}$ with
257 different Re numbers (5000 and 8293). Microbiological analysis showed that the support
258 material did not affect significantly biofilm growth. However, operating under continuous
259 flow (0.8-1.9 Pa) or stagnant water had a significant effect on biofilm formation: in stagnant
260 water the biofilm grew to a less extent. The same authors assessed how hydraulic conditions
261 (stagnation or flushing) can affect the biological stability of biofilms and evaluated the
262 relationship between the stability and the microbial composition of biofilms using a flow cell

reactor. Continuous turbulent (Re = 4900, 6 L.min-1) and laminar (Re = 810, 1 L.min-1) flow regimes were used and biofilm formation was monitored for 20 days. Afterwards, the system was subjected to unsteady hydraulic conditions (Manuel et al., 2010). Independently of the flow regime under which the biofilm was formed, stagnation promoted bacterial accumulation, either as attached or suspended forms, which were carried away in higher numbers when flow was re-started, thereby compromising the biological quality of the water. In all cases, Betaproteobacteria was the dominant phylogenetic group, although Gamma and Alpha subclasses were also present. These results suggest that special attention should be given to the biological quality of DW when consumption is subjected to strong variable demands (Manuel et al., 2010).

2.4. Propella® reactor

The Propella® reactor was already used by several authors for DW biofilms studies (Dailloux et al., 2003; Gosselin et al., 2013; Lehtola et al., 2006, 2007; Rubulis and Juhna, 2007; Simões et al., 2012; Torvinen et al., 2007). It consists of two concentric cylinders in which the propeller pushes the liquid down through the inner tube and then up through the annular section between both cylinders (Fig. 5). It is a perfectly mixed reactor and the fluid velocity, hydraulic residence time and the flow rate are controlled by the rotation speed of the propeller (Table 1). Coupons are usually located in the outer tube facilitating the sampling process and in some cases the removal of coupons does not change the flow conditions.

The ability of this reactor to simulate the process conditions commonly found in real DWDS makes it attractive for diverse studies. Dailloux et al. (2003) used a Propella® reactor with 2.08 L of volume (with high-density polyethylene (HDPE) coupons), water velocity of 0.2 $m.s^{-1}$, fed continuously with tap water (83.5 $mL.h^{-1}$) and inoculated with *Mycobacteria*

287 *xenopi* in order to evaluate the ability of this bacterium to colonize the experimental DW
288 biofilms. The authors verified that biofilms may be reservoirs for the survival of *M. xenopi*
289 and contributors to the continuous contamination of DW by erosion processes. Lehtola et al.
290 (2006) used *Mycobacterium avium* and a 2.3 L Propella® reactor with PVC coupons, working
291 at a flow rate of 183 $mL.min^{-1}$ (Re = 15000, retention time = 12.6 h). And they concluded
292 that this bacterium is able to survive and grow in DW biofilms and possibly transmitted via
293 DW. The same reactor and the same conditions were used in other study to assess the survival
294 of *M. avium, Legionella pneumophila* and *Escherichia coli* in DW biofilms under high-shear
295 turbulent flow conditions (Lehtola et al., 2007). This study clearly proved that pathogenic
296 bacteria entering DWDS can survive in biofilms for at least several weeks, even under
297 conditions of high-shear turbulent flow, and may be a risk to water consumers. This reactor
298 also was used to study the influence of phosphorus concentration on biofilm development
299 (Rubulis and Juhna, 2007; Torvinen et al., 2007). Rubulis and Juhna (2007) used the
300 Propella® reactor with PVC coupons fed with DW, at 0.25 $m.s^{-1}$ and retention time of 24 h,
301 aiming to assess the possibility to prevent biofilm formation by the removal of phosphorus.
302 Those experiments showed that removal of phosphorus to very low levels (< 1 $\mu g\ L^{-1}$) was
303 not an efficient strategy to eliminate bacterial regrowth and biofilm formation in DWDS.
304 Torvinen et al. (2007) studied the influence of low phosphorus concentration, flow rate and
305 temperature on the survival of *M. avium* in DW biofilms using a Propella® reactor with PVC
306 coupons (185 $mL.h^{-1}$; 0.24 $m.s^{-1}$; Re = 15000; 12.4 h of retention time). The authors
307 concluded that temperature is a more important factor than the availability of nutrients,
308 particularly phosphorus, on the survival of slow growing *M. avium* in DW biofilms. On the
309 other hand, an increase in water flow velocity had no effects on the survival of *M. avium*,
310 although it increased biofilm productivity.

2.5. Rotating Disc Reactor

The rotating disc reactor (RDR) consists in a tank with a rotating disc that is submerged in water (Fig. 6). The disc holds several coupons disposed concentrically and, as happens with the CCR, the shear forces depend on the rotational speed and on the diameter where coupons are allocated (Abe et al., 2011, 2012; Pelleïeux et al., 2012).

Abe et al. (2011) used this type of reactor to assess the elasticity and physico-chemical properties of DW biofilms in different stages of growth at constant hydrodynamic conditions (hydraulic shear stress of 0.12 Pa and shear rate of 120 s^{-1}). DW biofilms showed a spatially discontinuous and heterogeneous distribution comprising an extensive network of filamentous fungi in which biofilm aggregates were embedded. These results suggest that the DW biofilms were composed of a soft top layer and a basal layer with significant high elastic modulus values, falling in the range of fungal elasticity. The same authors used the RDR to study the cohesiveness and hydrodynamic properties of young DW biofilms (Abe et al., 2012). In this study the reactor was operated over three months at shear rates of 120, 175 and 230 s^{-1} (hydraulic shear stress of 0.120, 0.175 and 0.230 Pa, respectively), according to the location radius of each coupon. The results highlighted DW biofilm mechanical behavior depending on cohesiveness strength profile; the increasing of shear stress promoted a layer by layer (stratified structure) biofilm removal; and the detachment shear stress was weakly impacted by the biofilm age (from 4 to 12 weeks) and the hydrodynamic formation conditions (from 0.120 to 0.230 Pa). Pelleïeux et al. (2012) studied the accumulation of phages on DW biofilms at different shear rates (from 450 to 1640 s^{-1}) and under flow/non-flow conditions. All shear rates studied did not cause differences in the levels of virus and bacteria. However, convective diffusion (flow conditions) led to an increase of about 1 log in virus concentration

on surfaces compared to the levels of the pseudo-steady-state reached during the Brownian diffusion (non-flow conditions). The presence and behavior (survival) of some pathogens (*L. pneumophila, P. aeruginosa, Klebsiella pneumoniae* and *Flavobacterium sp.*) in DW biofilms also was studied by Murga et al. (2001) using the RDR with a flow rate at 1 mL.min^{-1}(residence time 6.7 h). It was found that, although unable to replicate in the absence of protozoa, *L. pneumophila* was able to persist in DW biofilms.

In RDR, as the entire disc rotates in the water, each radial position experiences a varying hydraulic shear stress, which enables the simultaneous formation of biofilms under different hydrodynamic conditions while keeping all the other conditions constant.

2.6. CDC biofilm reactor

The Centers for Disease Control (CDC) biofilm reactor, also known as CBR, was already used as a DWDS model. In this reactor the coupon holders are supported by a ported lid with each holder containing usually 3 coupons (Fig. 7). The lid with the holders is mounted in a vessel and the agitation is ensured by placing the reactor on a controlled stirrer plate, providing a constant rotation of the baffle (Armbruster et al., 2012; Goeres et al., 2005; Morrow et al., 2008; Park et al., 2012; Park and Hu, 2010). This reactor was used for different applications. Park and Hu (2010) used it to assess the effects of a reverse osmosis water pre-treatment on biofilm development in DWDS. However, this pre-treatment was unable to produce biologically stable water, although it had lower growth potential than the tap water produced from conventional water treatment. Armbruster et al. (2012) used a CBR to develop a stable, repeatable, DW multispecies biofilm model (*Sphingomonas paucimobilis*, *Methylobacterium* sp., *Delftia acidovorans*, and *Mycobacterium mucogenicum*) to investigate the interaction of the opportunistic pathogen *M. mucogenicum* with other DW

359 species, and determined the efficacy of monochloramine as a disinfectant (batch and
360 continuous flow disinfection) against two weeks old biofilms. The reactor operated under
361 batch mode (24 h, 100 rpm) followed by continuous flow conditions (2.5 $mL.min^{-1}$, 100 rpm,
362 140 min residence time, 13 d). Biofilms persisted in 1 $mg.L^{-1}$ monochloramine over 24 h but
363 detached bacteria suspended in DW were reduced. Although *M. mucogenicum* preferentially
364 resided in the biofilm, disinfectant exposure caused release of viable *M. mucogenicum* from
365 the biofilm into the water. DW biofilms were more tolerant to continuous flow disinfection,
366 which mimicked conditions found in distribution systems more closely than batch
367 disinfection. Morrow et al. (2008) used this device to investigate the impact of fluid shear on
368 *Bacillus* spores association with biofilm conditioned surfaces in DWDS and the subsequent
369 decontamination with chlorine and monochloramine. Biofilm associated spores required 5-
370 to 10-fold higher disinfectant concentrations to observe the same reduction of viable spores
371 as in suspension. Traditional chemical disinfection with monochloramine and chlorine was
372 an inappropriate strategy for decontamination of *Bacillus* spores from treated water systems.
373 These authors rationalized the selection of the CDC as DWDS model attending to the
374 possibility to control fluid shear on coupons surface (Morrow et al., 2008; Park and Hu,
375 2010).
376
377 **2.7. Microtiter plates**
378 The microtiter plates are nowadays the most frequently used reactor system for studying
379 biofilm formation. These can be used as a rapid and simple method to screen simultaneously
380 the effect of high numbers of different parameters on biofilm formation (Simões et al., 2007,
381 2010a, 2011). Simões et al. (2010a) used this device to study the adhesion and biofilm
382 formation on polystyrene by DW isolated bacteria (*Acinetobacter calcoaceticus*,

383 *Burkholderia cepacia, Methylobacterium sp., M. mucogenicum, Sphingomonas capsulata*
384 and *Staphylococcus sp*.). The overall results indicate that initial adhesion did not predict the
385 ability of the tested bacteria to form a mature biofilm, suggesting that other events (*e.g.*
386 phenotypic and genetic switching and the production of EPS) may play a significant role in
387 biofilm formation and differentiation. In other studies, Simões et al. (2007, 2010b) used
388 microtiter plates to assess biofilm interactions between DW isolated bacteria and the
389 influence of bacterial diversity on biofilm resistance to disinfection. In the first study, the
390 results shown that the parameters assessed by planktonic studies (growth rates, motility,
391 production of quorum-sensing inhibitors) did not allow prediction and generalization of the
392 exact mechanism regulating dual-species biofilm formation. Other cell-cell events, such as
393 intergeneric coaggregation, may play a significant role in the formation and interspecies
394 interactions in DW biofilms. Moreover, it was possible to identify synergistic, antagonistic,
395 and neutral interactions between DW bacterial biofilms. The other study allowed to conclude
396 that the bacterial diversity and their interactions may enhance biofilm resistance to
397 disinfection. The same device was also used by Simões et al. (2011) to investigate the effects
398 of metabolite molecules produced by these bacteria on their single and multispecies biofilms.
399 This study allowed the identification of bacterial species which have biocontrol potential (*M.*
400 *mucogenicum*) or have a significant role in development and maintenance of the DW
401 consortium (*A. calcoaceticus* and *B. cepacia*). These studies proposed that the elucidation of
402 the mechanisms by which diverse species survive and interact in DW biofilm communities
403 may allow the identification of new biofilm control strategies.
404 Gião et al. (2011) used this device to evaluate the interaction of *L. pneumophila* and
405 *Helicobacter pylori* with bacterial species isolated from DW biofilms and to study the
406 influence of different autochthonous microorganisms on the incorporation and survival of

these two pathogens in biofilms. *Mycobacterium chelonae* (pathogen commonly found in DWDS) seems to have a positive effect on the cultivability of both pathogens and seems to play an important role in the survival and control of these two pathogens in DW biofilms. This work also suggests that the presence of some microorganisms can decrease the cultivability of *L. pneumophila* but not the viability, which indicates that the presence of autochthonous microorganisms can lead to misleading results when the safety of water is assessed by cultivability-based methods alone.

This reactor has the obvious advantage of allowing high-throughput analysis, some of those can be non-invasive using microscopy (Bridier et al., 2013). However, the limitations to reproduce the environmental conditions found in a DWDS are significant.

2.8. Other bench top devices

Other laboratorial devices were developed to allow a better study of DW biofilm formation and control under specific conditions, in order to fill the gap on the limitations of existent reactors.

2.8.1. Flow chamber

The flow chamber is a simple device already used in DW biofilm studies. This allows a direct non-invasive observation of biofilm formation using microscopy. Paris et al. (2007) used four flow chambers fed in parallel with tap water and coupled to an inverted microscope to study the effects of different shear rates (34.9, 74.8, 142.5 and 194.5 s^{-1}) on biofilm development and structure. During the first stage of biofilm formation, bacterial accumulation was a function of the wall shear rate: the higher the wall shear rate, the faster the bacterial deposition. After 50 days, surface coverage was more or less identical for all wall shear rates,

suggesting that biofilm bacterial density cannot be controlled using hydrodynamics. However, the spatial distribution of the biofilm was clearly different. Under low wall shear rate, aggregates were composed of bacterial cells able to ‘‘vibrate’’ independently on the surface, whereas, under a high wall shear rate, aggregates were more cohesive. The same authors (Paris et al., 2009) used the same experimental setup described in the previous study with DW biofilms. The authors examined biofilms with two model particles recognized as hard (polystyrene) and soft particles (*E. coli*) in order to investigate the distribution and persistence of these allochthonous particles inoculated in DW flow chambers at various wall shear rates (70 to 460 s^{-1}) in biofilms with different ages (from 6 to 10 months old). The study showed that biofilm age (*e.g.* bacterial biofilm density and properties) and convective-diffusion governed the particle accumulation: older biofilms and higher wall shear rates both increased the velocity and the amount of particle deposition on the DW biofilm.

2.8.2. Reactors with glass beads

Bauman et al. (2009) described another device, a reactor containing glass beads, where the DW biofilms were developed and their ability to retain *E. coli* was analyzed. The study concluded that this engineered biofilm systems may be considered as a relevant device to capture pathogens from the bulk flow for monitoring purposes. So, it can contribute to improve the general insights into interactions between pathogens and DW biofilms. Codony et al. (2005) and Morato et al. (2005) used a packed-bed biofilm reactor filled with glass beads to monitor DW biofilm development by removing the biofilm attached to these beads for off-line analysis. Lehtola et al. (2002) used a PVC chamber covered with aluminum foil containing PVC slides to study the effects of low concentration of phosphorus in biofilm

development. The results showed that the availability of phosphorus regulated not only the development rate of biofilms but also microbial numbers during steady-state.

2.8.3. Chemostat

A two-stage chemostat model system was used to evaluate the persistence of *H. pylori* in DW biofilms (Gião et al., 2008). For biofilm formation, the chemostats were fed with filter-sterilized tap water at 50 $mL.h^{-1}$. These vessels contained PVC coupons used to sample the biofilm overtime. The influence of three parameters (low carbon concentrations, shear stress and temperature) on the persistence and cultivability of *H. pylori* in DW biofilms was studied. The results shown that shear stress did not influence negatively the numbers of *H. pylori* cells attached, suggesting that the autochthonous DW bacteria have an important role in retaining this pathogen in the sessile community.

Teng et al. (2008) used a simple system where coupons were submerged in a glass bottle fed with tap water. The cast iron coupons were removed at different times and after each sample the water was displaced with new tap water or sterile tap water to simulate the intermittent water flow environment in pipes. The aim of the study was to assess the effects of biofilms on cast iron pipe corrosion over time in DWDS, namely the characterization of corrosion scales and DW biofilm community structure. The authors demonstrated that the biofilm can greatly affect element composition and crystalline phase of corrosion scales. Also, biofilms accelerated corrosion in the first 7 d, but inhibited corrosion thereafter, which was due to the changes in the biofilm microbial diversity (presence of iron bacteria and iron reducing bacteria).

van der Kooij et al. (1995) designed a device based on two principles: the hydraulic conditions should resemble those in pipes of real systems, and should have a simple

construction and use. The device consisted in a vertically placed glass column, containing 40 glass or Teflon cylinders for cell adhesion. The water flowed downward through the column (4.6 $L.min^{-1}$, 0.2 $m.s^{-1}$) coming in contact with the inner and the outer surface of the cylinders. With this system, the authors assessed the effects of support material, water type and nutrients on the rate and extent of biomass accumulation. The results showed that the material type (glass and Teflon) and the cylinder position had minor or insignificant effects on biomass accumulation. On the other hand, biofilm formation was strongly enhanced by low concentrations of easily available substrates, such as acetate.

2.8.4. Glass ring column

A glass ring column device, similar to the flow cell system, was used to assess the influence of biofilms on Fe and Mn deposition in DWDS (Ginige et al., 2011). The column was feed with tap water inoculated with DW microorganisms, namely *Pseudomonas fluorescens* and *Spirillum* spp. The reactor was allowed to operate continuously for 4.5 months at a flow rate of 0.42 $mL.min^{-1}$ and a recirculation rate of 667, the reactor resembled a completely mixed flow-through configuration. This study addressed the contribution of biofilms to discoloured water incidents. Biofilms facilitated the deposition of Fe and Mn on pipe walls, an increase in biofilm activity was associated with an increase in Fe and Mn accumulation. So, reducing biofilm accumulation should be considered along with other strategies, such as removal of Fe and Mn *via* water treatment to better manage discoloured water events.

2.8.5. Pedersen device

The Pedersen device is used coupled to other bench top devices, as flow cells, and it was used to study biofilms in flowing-water systems (Pedersen, 1982). To build this device,

502 microscope cover slips were fitted into acrylic plastic holders forming two parallel test piles,
503 each with room for 19 slips. The test piles were placed in flow cells, and in order to separate
504 the flow at the inlet of the reactor three diffusers with different hole patterns were used. These
505 diffusers were located in both sides of the reactor, being possible to change the flow direction.
506 Flow stabilizers, which were identical to test piles, were used to establish a laminar flow
507 between the slips. The sampling process in this device was done at fixed times taking out a
508 desired number of slips for off-line analysis. Normally, one sample consisted of two slips,
509 one from each of the two parallel piles. The sampled slips were replaced with new ones in
510 order to maintain the flow conditions (Pedersen, 1982).
511
512 *2.8.6. Loop with biofilm test-plug module*
513 Boe-Hansen et al. (2003) developed a loop with biofilm test-plug module in order to simulate
514 DWDS conditions and to produce a large number of biofilm samples grown under
515 comparable conditions. This device was constituted by two identical loops connected in
516 series and in each loop there was an adjustable centrifugal pump to recycle the water. The
517 recycle-flow rate was controlled by a needle valve installed immediately downstream the
518 pump. Both loops contained two strings of biofilm test-plug modules made from square
519 pipes, each string consisted of a row of 5 biofilm test-plug modules, each with 7 test-plugs.
520 To prevent turbulence induced by pumps, valves and bends and to stabilize the flow inside
521 the modules, a 2 m square pipe was inserted just upstream of the test plug modules. This
522 construction should guarantee that the velocity distribution was identical from cross-section
523 to cross-section, and that all test plugs within a loop were exposed to identical hydraulic
524 conditions. Using this device, Boe-Hansen et al. (2003) monitored biofilm formation and
525 activity in DWDS under oligotrophic conditions. The purpose of this study was to test 11

526 different microbial methods for monitoring biofilm in DW, at low nutrient conditions. The
527 methods used allowed biofilm characterization in terms of biomass quantification, metabolic
528 activity measurement, structure visualization and microbial diversity profiling. The model
529 distribution system was continuously fed with DW from a municipal distribution network
530 (retention time 2 h, flow velocity 0.07 $m.s^{-1}$). The model distribution system and the biofilm
531 sampling modules used in this study provided an easy access to a large number of biofilm
532 samples. The system allowed biofilms to be grown under controlled conditions comparable
533 to those prevalent in the DWDS. The retention time, the flow rate and temperature were
534 independently controlled in the system, and furthermore it allowed chemicals or specific
535 microorganisms to be added.
536
537 **3. *In situ* application devices**
538 The *in situ* devices were developed to study and monitor DW biofilms in pilot and real
539 DWDS. These devices are usually placed as a by-pass or directly connected to a DWDS.
540
541 **3.1. Robbins device**
542 The Robbins device is one of the mostly used to study biofilm behavior *in situ* in real and
543 pilot scale DWDS. The Robbins device is a pipe with several threaded holes (Fig. 8). Some
544 screws with coupons mounted on the front side are placed in these holes (Manz et al., 1993;
545 Sly et al., 1990). The coupons are aligned parallel to the water flow and can be removed
546 independently (Manz et al., 1993).
547 As referred previously, the Robbins device can be applied directly to real DWDS. Sly et al.
548 (1990) studied the manganese deposition in a DWDS in the Gold Coast (Australia). The
549 results showed that manganese (bulk concentration of 0.05 $mg.L^{-1}$) deposition occurred by

chemical and microbial action, although the chemical deposition rate was much higher than microbial deposition. Manz et al. (1993) also used the Robbins device to test biofilm formation on glass slides in the Norrvatten (Sweden) DWDS at a distance of 30 km from the waterworks. These authors found that surface-attached cells are more active than free-living equivalents. Also, the authors found that microcolonies in very early stages of development consisted of mixed populations.

As the Robbins device is responsible for significant changes of the water flow on the slides, several authors developed a modified Robbins device (MRD). Nickel et al. (1985) developed a MRD to assess the degree of resistance of biofilm bacteria to antibiotics in catheter material. This new device consisted in a pipe with 25 spaced sampling ports attached to sampling plugs flushed with the inner surface, without disturbing the flow characteristics. Kalmbach et al. (1997) used the MDR in a DWDS of Berlin (Germany) with a flow rate near of 6 $L.h^{-1}$ to investigate the metabolic activity and the phylogenetic affiliation of single adherent bacteria during colonization and biofilm formation in DW. The authors found that respiratory activity of adherent bacteria decreased continuously during the early stages of biofilm formation. Carter et al. (2000) used this device in the Milford (USA) DWDS using a flow rate near of 0.4 $L.min^{-1}$. The main goal of this study was to identify relationships among heterotrophic bacteria and standard physical and chemical water quality parameters. A relationship was found particularly to cultivability counts on R2A medium. Silvestry-Rodriguez et al. (2008) also used this device to study biofilm control in an experimental plant using water from Tucson (USA) DWDS, operating at 0.4 $L.h^{-1}$. PVC and stainless steel were used as biofilm formation substrate, however, no significant inactivation was observed on both surfaces when treated with silver at 100 $\mu g.L^{-1}$.

573 Latter, Kerr et al. (2000) developed the newly modified Robbins device (nMRD) that
574 consisted in a MRD adapted to form two separate halves, being possible to take it apart and
575 to clean it. This new device was constructed from Perspex and the two separate halves were
576 held together by thirty screws, and the whole device had Perspex connectors at both ends to
577 which the tubes were attached. This study was performed in order to investigate the
578 reproducibility of attachment and whether there was a statistical significant gradient of
579 adhesion along the 25 sampling ports of the nMRD. No significant difference occurred
580 between pairs of nMRDs that were run in parallel, however, there was a significant difference
581 between different batches of bacteria. It also was observed that the position of the sample
582 disc influenced bacterial adhesion. Other variation of the Robbins device was presented by
583 Jass et al. (1995) that used a chemostat-coupled MRD. The association of a chemostat and a
584 MRD provides a large number of sample surfaces for monitoring biofilm formation and
585 control over extended periods of time. These authors proposed that this device can be
586 successfully used for studying bacterial adhesion and biofilm formation in tubular devices.
587

588 **3.2. Pennine Water Group coupon**

589 Recently, it was developed a new coupon sampling device for *in situ* studies, the Pennine
590 Water Group (PWG). This coupon can be inserted directly into the pipes of DWDS,
591 maintaining flow conditions representative these near wall pipe and enabling simultaneous
592 quantitative and qualitative compositional characterization of *in situ* biofilms (Deines et al.,
593 2010). This offers improvements over alternative sampling devices and the coupons are
594 comprised of two parts, an “outer coupon” and an “insert” (Fig. 9). The outer coupon retains
595 the curvature of the pipe and fits precisely into a hole made in a removable and flanged
596 identical pipe section. The coupon is fixed with a gasket to a section pipe. The insert is

597 engineered flat to allow microscopic analysis and it fits inside of the outer coupon in a way
598 to allow the outer surface to be in direct contact with the water. This design has a maximum
599 deviation from curvature of 0.064 mm, in the order of magnitude of the surface roughness
600 coefficient used in hydraulic models (Deines et al., 2010). It is an accurate device and allows
601 direct insertion and close alignment with the internal pipe surface, minimizing the distortion
602 of boundary layer conditions that influence biofilm formation, such as boundary shear stress
603 and turbulent driven exchange with the bulk water body (Douterelo et al., 2013). This coupon
604 was used in a full-scale laboratory pipe loop. Deines et al. (2010) used a constant flow rate
605 of 0.4 $L.s^{-1}$ (boundary shear stress of 0.03 $N.m^{-2}$) and it was observed an increase in bacterial
606 biofilm coverage of the coupon surface over time, as well as, the development of increasingly
607 complex biofilm communities. Douterelo et al. (2013) used PWG coupons to evaluate the
608 effect of different and variable flow rates (0.2 to 0.5 $L.s^{-1}$; 0.2 to 0.8 $L.s^{-1}$ and 0.4 $L.s^{-1}$) on
609 biofilm development and detachment from pipe walls. They concluded that different
610 hydraulic regimes affect the composition and diversity of bacterial communities in biofilms.
611 However, the use of increasing flow rates did not completely remove bacteria from pipe
612 walls.
613

614 **3.3. Bioprobe monitor**

615 The bioprobe monitor was specifically designed to study biofilm growth within a pipe
616 system. LeChevallier et al. (1998) described a pilot-scale DWDS (1.3 km) that had an
617 experimental test station with 24 m and contained three test sections. A bioprobe monitor
618 was located at the beginning of each experimental section to monitor the environmental
619 conditions and biofilm development. The bioprobe monitor consists of a pipe where it is
620 inserted a coupon holder (denominated acetal) being the coupon surface flushed with the pipe

621 wall (Fig. 10). LeChevallier et al. (1998) also used this device to study the effects of chlorine
622 and monochlorine on biofilm development at a water flow rate of 0.07 $L.s^{-1}$. These authors
623 observed that the density of bacteria on the iron surfaces reached a maximum when the
624 temperatures were higher and when there was a total declination of chlorine residuals. Also,
625 they observed lower cell densities in the first section of the pilot-scale DWDS and this was
626 due to the fact that more chlorine reached this part of the system.
627

628 **3.4. Other *in situ* devices**

629 Other devices were used for *in situ* DW biofilm studies. Juhna et al. (2007) used a biofilm
630 sampler that consists in a coupon holder inside of a pipe section. The authors used a total of
631 22 holders exposed to DW in a DWDS from Latvia and France to detect *E. coli*. This
632 bacterium was found in 56% of the coupons using peptide nucleic acid fluorescent *in situ*
633 hybridization (PNA-FISH), however, it was not detected using culture-based or enzymatic
634 methods. The presence and amount of *E. coli* detected was not correlated with any physical
635 and/or chemical characteristics of DW such as the temperature, chlorine or biodegradable
636 organic matter (BOM) concentration. Helmi et al. (2010) used a pilot device constituted by
637 5 PVC compartments comprising a holder with six removable discs allowing the study of the
638 effects of different surface materials on biofilm development. The device was connected to
639 the tap of a DWDS operating at a flow rate of 2 $L.min^{-1}$ in order to study the interaction
640 between virus and DW biofilms and to develop a method to detect viral particles in these
641 biofilms. Five protocols were used for viral recovery, testing different sonication intensities
642 (20% and 40% power intensity) and its combination with centrifugation (1500 g for 10 min)
643 and with pH neutralization. The most efficient protocol, that combined all the steps, allowed
644 a recovery rate from 29.3% to 74.6% depending on the virus and on the material. The study

of viral interactions with DW biofilms allowed to conclude that viral adsorption to biofilms depends on their isoelectric point, the disc material and the hydrodynamic conditions. For example, the viral adsorption to biofilms is less than 1% of the initial viral load when hydrodynamic conditions similar to those existing in DWDS were applied. Prévost et al. (1998) developed a study using a biofilm coupon device, known as the Prévost device. This device was installed on two DWDS of the city of Laval (Canada) and was used to remove the biofilm samples from the DWDS. The authors installed diverse devices in valve chambers and investigated the impact of nutrients levels and oxidant residual maintenance in the biofilms formed in the DWDS. They found that a low nutrient concentration reduced bacterial biomass. Nevertheless, the most significant differences were only observed in warm water and not in cold water.

Another device is the sliding coupon holder, a pilot-scale device (Chang et al., 2003). This device is a half PVC pipe where coupons are located, being easily removed and replaced after each experimental phase. Chang et al. (2003) used this device to determine the effects of blending different water qualities on the final quality of the water in the distribution system. The biofilm density was estimated on different pipe materials using a specific DNA-probe (BO-PRO^{TM} 3). They concluded that this technique provided results that were correlated to these obtained from heterotrophic plate counts on R2A medium, after biofilm scrapping. Therefore, the technique used allowed to quantify fixed biomass without disrupting the biofilm.

Långmark et al. (2005) investigated the accumulation and fate of a model microbial pathogen in natural grown biofilms formed in a pilot-scale DWDS provided with chlorinated and UV-treated water. Two pilot-scale DWDS were used, comprising 1 km of polyethylene tubing that was connected directly to the finished water. The biofilm sample devices were chambers

669 equipped with 20 exchangeable glass slides and were located at various distances along each
670 DWDS pilot scale, corresponding to different residence times (0.1, 15, 40 and 110 h) within
671 the main Stockholm DWDS. It was not found a significant impact of primary disinfection
672 processes on the accumulation and fate of pathogen models (*L. pneumophila* and
673 bacteriophages) within the DWDS.
674 Other devices were constructed to study microfungal behavior in DWDS. Sammon et al.
675 (2011) investigated the microfungi colonization of hard surfaces within the storage and
676 distribution system by suspending artificial coupons within the water body of reservoirs.
677 Sammon et al. (2011) used glass, PVC and concrete coupons held in sets of custom-made
678 racks. These racks were designed to held one coupon vertical and apart from the other
679 coupons, to ensure a free flow of water across both surfaces of all coupons. The racks were
680 placed in lidded plastic basket which was perforated on all sides, bottom and top. The basket
681 was attached to nylon ropes and a clay house brick was used to held the basket at 1.5 m from
682 the bottom. This work allowed to conclude that airborne spores introduced into reservoirs
683 can be an important external source of microfungal propagules, however, it was also observed
684 that the microfungi were not involved in the primary colonization of surfaces. The results
685 also suggested that any aggregation of soft sediment in the DWDS was a potential site for
686 the proliferation of the microfungal population. Siqueira et al. (2011, 2013) proposed the use
687 of a distinct device to investigate natural filamentous fungi biofilms in DWDS, the sampler
688 device. The core of the sampler device consists of hollow PVC pipes with polyethylene or
689 acetate coupons held in place to allow biofilm growth. The end of each sampler forms a screw
690 to connect multiple samplers or to close the device with a cap after coupon removal from the
691 water network. These features facilitated insertion, handling and removal of each sampler
692 device after collection and preventing contact with external environment during the transport

process. Finally, the pipes could be filled with water in order to maintain moisture and preserve the integrity of the biofilms formed on the coupons. Siqueira et al. (2013) used this device in a DWDS at Recife (Brazil), concluding that this device is useful to study DW biofilms and that Calcofluor White (CW) staining is a rapid and efficient method to detect filamentous fungi, allowing its differentiation by morphology. This study also demonstrated that fungi are likely to play an important role in DWDS biofilms and final water quality.

The main advantages and limitations of the main devices described previously are synthesized in Table 2. These are mostly related with the ability to study and control the hydrodynamic conditions, with the sampling process, the temperature control, the possibility to use different surface material, and the possibility to operate under conditions similar to the real systems.

4. Main applications of reactors in DW biofilms studies

In general, the main applications of several described reactors in studies of DW biofilms are monitoring the biofilm formation with different operational conditions (support material, hydrodynamics, temperature, nutrients, type of microorganisms, disinfectants) and biofilm control by different strategies (process conditions and disinfection). Table 3 synthesizes some of the studies on DW biofilms using reactors, making reference to the main process conditions and microorganisms used.

4.1. Biofilm control

Several strategies can be used to attempt biofilm prevention and control in DWDS. The pre-treatment of water, before being released into the DWDS is an important preventive measure

and usually consists in the minimization of the organic matter and nutrients concentration entering the distribution system. The material selection to apply in the DWDS pipes and fittings is also important to control biofilm development. The use of antimicrobial compounds is common, being important to maintain a residual concentration of disinfectant inside the DWDS. Simões and Simões (2013) described usual and new techniques used to prevent and control biofilm formation in DW. Nonetheless, biofilm control by manipulating the operation conditions (temperature, flow rate and shear stress, presence of nutrients, material selection) is also a matter of study (Ndiongue et al., 2005; Rickard et al., 2004; Simões et al., 2006; Torvinen et al., 2007).

4.1.1. Management of operational conditions

To control biofilm development it is important to understand how its development happens and the role played by the operational conditions (Douterelo et al., 2013; Lehtola et al., 2007; Pintar and Slawson, 2003; Simões et al., 2006; Torvinen et al., 2007; Volk and LeChevallier, 1999). Ollos et al. (2003) evaluated the influence of several factors (BOM concentration, monochloramine and chlorine disinfection, flow velocity, pipe material and temperature) on biofilm development using as DWDS model an annular reactor. Under the conditions studied, the disinfectant residual was the most important factor for biofilm accumulation. In the absence of BOM, temperature seemed to have no effect, whereas shear stress seemed to be important. In the presence of BOM, temperature was important at low shear stress, although shear stress conditions themselves had little effect. The condition leading to the strongest biofilm accumulation was a high level of BOM combined with the absence of a disinfectant. The temperature effect was studied by Ndiongue et al. (2005) and Pintar and Slawson (2003) using an annular reactor, as previously referred. Torvinen et al. (2007), as already said, used

741 a Propella® reactor to assess the effects of different temperatures on biofilm growth, but also
742 studied the influence of flow velocity and phosphorous concentration.
743 The effect of hydrodynamic conditions was investigated in biofilm growth using diverse
744 reactors. The flow cell system is one of the systems used to achieve this goal (Manuel et al.,
745 2010; Simões et al., 2006), as well as the Propella® reactor (Lehtola et al., 2007). CCR and
746 RDR allowed the evaluation of the effect of different shear stresses on biofilm development
747 (Abe et al., 2012; Rickard et al., 2004). The *in situ* devices also can be used to study the
748 hydrodynamic effects on biofilm development, simulating a flushing situation, as did by
749 Douterelo et al. (2013) using the PWG coupon, as previously referred.
750 Another important aspect that can help to control biofilm development is the type of surface
751 material. The annular reactor was expressively used with this aim. Zhou et al. (2009) used
752 this device to study the effects of surface material (SS and copper - Cu) on disinfection by
753 chlorine and chloramines. The results showed that biofilm formation was affected either by
754 the type of disinfectant as well as by the type of pipe material. Chloramines were more
755 effective than chlorine in controlling biofilms formed on both SS and Cu surfaces. The tested
756 pipe materials did affect bacterial accumulation when chlorine and chloramines were present.
757 There were fewer bacteria attached to Cu slides with chloramines or chlorine disinfection
758 when compared with SS. The combination of Cu pipes and chloramines as the disinfectant
759 was the most efficient combination to get low biofilm accumulation. Jang et al. (2011) did a
760 similar study comparing the influence of steel, SS, Cu and PVC on biofilm formation and
761 water quality. An annular reactor with coupons of these materials was operated under
762 hydraulic conditions similar to a real plumbing system (50 rpm, 0.25 $N.m^{-2}$, approx. 0.3 ms^{-1}
763), at a flow rate of 170 $mL.min^{-1}$ for 15 months. The results showed that biofilm formation
764 and water quality were substantially affected by the pipe materials. The bacterial

765 concentration and species diversity in the biofilms increased with the corrosion of the pipe.
766 The bacterial accumulation was 100 times higher on steel pipe than on the other pipe
767 materials. SS demonstrated to be the best material among those tested, with the lower levels
768 of attached cells.
769 The control of nutrients in water can be used to mitigate biofilm formation. In order to
770 ascertain the influence of this parameter in biofilm formation, some authors used pre-
771 treatment strategies to remove the nutrients of real tap water, while others used synthetic
772 water with different nutrient concentrations. Reverse osmoses (RO) is one of the methods
773 used and can improve the water quality by reducing organic, inorganic and bacterial contents
774 (Simões and Simões, 2013). Park and Hu (2010) compared biofilm growth in a CDC reactor
775 fed with real tap water and fed with tap water previously treated through RO. The Propella®
776 reactor was used to prevent biofilm formation by controlling phosphorus concentration
777 (Rubulis and Juhna, 2007). The annular reactor was also used to study the influence of
778 nutrients in biofilm growth. Chandy and Angles (2001) and Fang et al. (2010) used this
779 device to determine the impact of nutrient limitation on biofilm growth and disinfectant
780 decay. The first study found that biofilm development was limited by organic carbon and that
781 biofilm development promoted chloramine decay. The removal of nutrients resulted in stable
782 chlorine persistence, which led to higher biofilm control. The authors proposed that the
783 treatment and operational management strategies should incorporate organic carbon removal
784 to limit biofilm development through a combination of retarding bacterial growth and
785 enhancing disinfectant persistence. Fang et al. (2010) developed DW biofilms in this device
786 to examine the effects of phosphorus on disinfection with free chlorine and monochloramine.
787 The disinfection efficacy was increased by phosphorus addition. The presence of phosphorus
788 was found to increase the biofilm cell numbers but decreased EPS production. At the same

disinfection dosages, monochloramine showed greater biofilm removal efficiency than free chlorine. These authors proposed that monochloramine could be a better choice than free chlorine in DW biofilm disinfection, when phosphorus is added as the corrosion inhibitor.

4.1.2. Disinfection strategies

Even if chlorine is the chemical agent most widely used for DW disinfection, studies are still performed to optimize disinfection strategies and to find alternative solutions. The annular reactor was used in several studies to evaluate different control strategies and the frequently tested disinfectants were those chlorine-based, as free chlorine, monochloramine and chlorine dioxide (Batté et al., 2003b; Chang and Craik, 2012; Dykstra et al., 2007; Gagnon et al., 2004; Murphy et al., 2008; Pintar and Slawson, 2003; Rand et al., 2007). However, other strategies were tested, including ozone (Chang and Craik, 2012), the combination of UV treatment with free chlorine, monochloramine and chlorine dioxide (Dykstra et al., 2007; Murphy et al., 2008; Rand et al., 2007). Fenton reaction was tested in a Propella® reactor by Gosselin et al. (2013). Morrow et al. (2008) and Hosni et al. (2011) used a CDC reactor to developed disinfection strategies also based in chlorine derivative disinfectants and UV treatment. Armbruster et al. (2012) considered that the comprehension of the extent of interaction between opportunistic pathogens with biofilms is needed to understand their role in DWDS. These authors used a CDC reactor to develop a multispecies biofilm and tested the disinfection efficiency of monochloramine. Silvestry-Rodriguez et al. (2008) studied the effect of silver on biofilm disinfection using a MRD, as previously referred.

4.2. Biofilm monitoring

Another strategy to understand biofilm formation and behavior is by their monitoring. The best devices for biofilm monitoring are those that have removable coupons, allowing the assessment of the gradual biofilm development and the changes during all formation stages. The use of appropriate coupons is also an important issue because the monitoring of the heterogeneous distribution of the biofilm over the surface area of the reactors is difficult due to the size and the shape of the surface (Okabe et al., 1995).

Simões et al. (2006) used a flow cell system to monitor biofilm development under different operational conditions (shear stress, support material and nutrients). Torvinen et al. (2007) used the Propella® reactor to follow the influence of flow velocity, phosphorus concentration and temperature on the survival of *M. avium* in biofilms. A similar application of Propella® reactor was done by Lehtola et al. (2006). Manuel et al. (2007) used both previously described reactors to monitor and evaluate how the dynamic conditions affected the stability of biofilms.

The RDR was used to monitor *L. pneumophila* survival on biofilms during 15 days (Murga et al., 2001). Pelleïeux et al. (2012) used a similar device to monitor the accumulation of enteric viruses on surfaces within a DWDS.

The annular reactor is often used to monitor DW biofilm development (Bachmann and Edyvean, 2006; Schaule et al., 2007; Zhang et al., 2010). Zhang et al. (2010) monitored the presence of heterotrophic and ammonia oxidizing bacteria (AOB) in biofilms to determine the potential relationship between the abundance of heterotrophic bacteria and of AOB, using an annular reactor. Bachmann and Edyvean (2006) used this device to study biofilm development of *Aquabacterium commune* on SS. Schaule et al. (2007) used an annular reactor linked to three sensors to gather information on the biofilm cell density. Even if this

device allows online monitoring, the existence of coupons is essential for microbiological characterization.

The use of biofilm-forming devices as a by-pass or directly connected to a DWDS has been a commonly used strategy to allow a more efficient monitoring of biofilm formation in pilot and real systems (Hallam et al., 2001). Sly et al. (1990) used the Robbins device to monitor the deposition of manganese in the presence of biofilms. Silvestry-Rodriguez et al. (2008) used the same device to monitor the effects of silver in biofilm control using tap water in an experimental plant.

Deines et al. (2010) and Douterelo et al. (2013) used the PWG device to study the diversity of biofilm communities within DWDS, as previously referred.

5. Quantification of biofilms in DWDS models

All the biofilm studies require the definition of an appropriate method to quantify biofilm formation and to provide information on its characteristics, particularly for the resident population. Biofilms can be quantified through the increase of biological activity or by the number of cells (Liu et al., 2013). Apart from the quantification of cell numbers, it is also important to obtain information on other biofilm constituents, particularly the EPS. Most of these methods require the biofilm scraping from the substratum and its dispersion in an adequate solution, generally saline water (Fang et al., 2009; 2010; Manuel et al., 2007; Park et al., 2012; Silvestry-Rodriguez et al., 2008; Zhou et al., 2009) or an appropriate buffer (Chang and Craik, 2012; Jang et al., 2012). Moreover, to achieve an efficient biofilm dispersion in the selected solution it is necessary to use some physical treatment as vortex and/or ultrasonication (Chang and Craik, 2012; Fang et al., 2009; 2010; Jang et al., 2012; Manuel et al., 2007; Park et al., 2012; Silvestry-Rodriguez et al., 2008; Zhou et al., 2009).

The exceptions to the scraping requirement are some microscopic methods, as atomic force microscopy (AFM), scanning electron microscopy and confocal scanning laser microscopy (CSLM), which can allow a direct analysis of biofilm adhered to a surface, if the sampling coupons are flat (Abe et al., 2012; Fang et al., 2010; Jungfer et al., 2013; Ling and Liu, 2013; Mathieu et al., 2014). However, even if the direct microscopic analysis of coupon surfaces is important to provide information on the biofilm structure, these methods cannot determine all relevant aspects involving the biofilm formation process. Therefore, the combination of information from different methods will provide a more detailed picture on DW biofilm formation and composition.

5.1. Cell enumeration

The biofilm quantification through cell enumeration is the mostly used method. The biofilm development and dynamics is commonly monitored through the enumeration of cultivable, metabolic active, viable and/or total cells (Chang and Craik, 2012; Fang et al., 2009; 2010; Gagnon et al., 2005; Jang et al., 2012; Jungfer et al., 2013; Manuel et al., 2007; Park et al., 2012; Silvestry-Rodriguez et al., 2008).

Heterotrophic plate count (HPC) methods are often used to assed the numbers of cultivable bacteria, usually described in terms of colony forming units (CFU) per unit of surface area. These methods only enumerate a fraction of heterotrophic bacteria on an agar-based medium under defined incubation temperature and time. To quantify the HPC, it is necessary to scrap the biofilm from the reactor/coupon surface and dilute it to an adequate concentration, before plating. This is a method often used to evaluate biofilm cell numbers in several DWDS models, as the annular reactor (Batté et al., 2003a; Gagnon et al., 2005; Zhou et al., 2009),

882 CDC reactor (Park et al., 2012), flow cell, Propella® reactor (Manuel et al., 2007) and the
883 MRD (Silvestry-Rodriguez et al., 2008).
884 The microbial metabolic active and total cell numbers are usually assessed through
885 microscopic analysis after a staining process and the results are usually represented in terms
886 of numbers of cells per unit of surface area. 4', 6 - diamidino - 2 - phenylindole (DAPI) or
887 acridine orange are common dyes used for total cell counts (Percival et al., 1998; 1999; Boe–
888 Hansen et al., 2002; Batté et al., 2003a; Gagnon et al., 2005; Juhna et al., 2007; Manuel et
889 al., 2007; Park et al., 2012). DAPI is a fluorescent stain that binds to A-T rich regions in
890 DNA fluorescing blue, and since it is able to pass through the cell membrane it stains both
891 live and dead cells. Acridine orange is a cell-permeable fluorescent stain that interacts with
892 RNA and DNA fluorescing green to red, providing information on the numbers of total and
893 viable cells (Yu et al., 1995). The *Bac*Light Live/Dead (L/D) stains provide a bacterial
894 viability kit that allows the assessment of both viable and total bacterial cell counts. This kit
895 is composed of two nucleic acid binding stains: SYTO 9 and propidium iodide (PI). SYTO
896 9 penetrates all bacterial membranes and stains the cells green, while PI only penetrates cells
897 with damaged membranes, and the combination of the two stains produces red fluorescing
898 cells (Simões and Simões, 2013). These stains interact with all the existing biofilm bacteria
899 and their quantification is processed by epiflourescence microscopy. Metabolic active
900 bacteria are usually assessed after being stained by the redox dye 5 - cyano-2, 3-ditolyl
901 tetrazolium chloride (CTC) which produces a fluorescent precipitate when it is intracellularly
902 reduced by respiring bacteria (Jungfer et al., 2013; Sierack et al., 1999). This method was
903 used by Gagnon et al. (2005) in an annular reactor, by Manuel et al. (2007) in the Propella®
904 reactor and in the flow cell system, and by Boe-Hansen et al. (2002) in its loop with biofilm
905 test-plug module.

FISH is a procedure used to identify and quantify certain bacteria species within the biofilm community. It consists in the use of fluorescent probes that bind specifically to a nucleic acid sequence. It was used by Park et al. (2012) to investigate the presence of a bacterial species within a biofilm formed in a CDC reactor.

5.2. EPS quantification

EPS have a determinant role in biofilm formation and physical stability. They are composed of a variety of organic substances and carbohydrates are its predominant constituents, whereas proteins also exist in substantial quantities. Therefore, the EPS quantification methods are usually based on the analysis of carbohydrates and proteins. However, the reliability of the analysis is strongly dependent on the extraction methods used to separate the EPS from the biofilm cells (Wingender et al., 1999). To quantify the carbohydrates it is often used the modified phenol-sulfuric acid method (Chandy and Angles, 2001; Fang et al., 2010; Percival et al., 1998; 1999). The carbohydrates are broken down by the concentrated sulfuric acid to monosaccharides. Pentoses are then hydrated to furfural and hexoses to hydroxymethyl furfural. These compounds react with phenol and produce a yellow–gold color with a maximum absorption at 490 nm (Dubois et al., 1956).

For proteins quantification Chandy and Angels (2001) quantified EPS proteins with a protein dye (Comassie Brilliant Blue). This dye is able to combine with proteins and their amount can be determined spectrophometrically at 595 nm.

5.3. Microscopic analysis

Some microscopic analyses are non-destructive, which means that it allows the direct observation of biofilms without a scraping step. These methods can be advantageous since

930 the possibility of biofilm loss in the scraping process does not exist, but also it allows the
931 study of the entire biofilm structure.
932 AFM is one of these methods and it provides topographic images from the micro– to the
933 nano-scale, providing qualitative and quantitative information on the physico-chemical
934 properties of biofilm-substratum interactions (Beech et al., 2002). Abe et al. (2012) and
935 Mathieu et al. (2014) used this method to study the biofilm behavior in a RDR. Abe et al.
936 (2012) applied AFM techniques, as nano-indentation and chemical force spectrometry, in
937 order to investigate the physico-chemical properties at different formation steps and ages of
938 DW biofilms. The nano-indentation experiments were used in order to investigate the
939 possible presence of macromolecules within a conditioning layer and its contour lengths
940 (maximal extension length of a polymer chain). Chemical force spectrometry was used to
941 assess the substratum and biofilm hydrophobicity. Mathieu et al. (2014) also used AFM to
942 study biofilm cohesiveness through the evaluation of the volume of clusters. To achieve this
943 goal, the surface area of each biofilm aggregate found on the scanned region was analyzed
944 through the corresponding AFM height image. These images were adjusted and treated with
945 a procedure scripted in MATLAB. The program returns the number of biofilm aggregates
946 present in the scanned region, and the surface area and volume of each aggregate.
947 Another non-destructive microscopic technique is the CSLM. This is a high-technology
948 epifluorescence microscope that creates a thin plane-of-focus, in which out-of-focus light is
949 eliminated (Palmer and Sternberg, 1999). It was used in several works to study DWDS
950 biofilm formation (Fang et al., 2009) and its behavior to disinfectant action (Fang et al., 2010;
951 Ling and Liu, 2013), where annular reactors and CDC reactors were used as DWDS models.
952 The CSLM allows analyzing the biovolume (spatial size) and the average thickness of
953 biofilms. These both parameters indicate the biofilm amount (Fang et al., 2010; Ling and

954 Liu, 2013). However, to assess these values, the use of fluorescence dyes is essential as the
955 combination of SYTO 9 and propidium iodine to stain cells (The *Bac*Light viability kit) and
956 lectin probes to visualize the biofilm EPS (Fang et al., 2009, 2010; Ling and Liu, 2013).
957

958 ### 5.4. Other quantification methods

959 The adenosine triphosphate (ATP) assay is a rapid approach with low detection limits (as low
960 as 0.0001 nM, < 5% deviation) for the indirect assessment of the number of viable cells (Liu
961 et al., 2013). ATP is converted to a luminescent signal (light) in the presence of a combination
962 of a substrate and an enzyme, luciferin and luciferase, respectively. This reaction is called
963 the luciferase reaction in which the mono-oxygenation of luciferin is catalyzed by luciferase
964 in the presence of Mg^{2+}, ATP, and molecular oxygen. The amount of luminescent signal
965 produced is proportional to the amount of ATP present which corresponds to the number of
966 viable cells (Wadhawan et al., 2010). Boe-Hansen et al. (2002) used this technique in the
967 developed loop with biofilm test-plug. This technique was used in DW biofilms to estimate
968 the size and activity of the microbial community. These authors used another method to
969 assess the biofilm formation. It consists in the incorporation of leucine to estimate the protein
970 synthesis rate as a measure of the bacterial growth, after a biofilm dispersion step. Leucine
971 was radioactively labeled and its incorporation was measured by scintillation.
972 Batté et al. (2003a) used an annular reactor to formed DWDS biofilms and estimated the
973 impact of phosphate-based corrosion inhibitors and the age of biofilm on bacterial cell
974 density using a potential exoproteolytic activity (PEPA) method. This method is used to
975 assess the potential of bacterial cells to hydrolyze proteinic organic matter. It consists in the
976 addition of L-Leucine-β-Naphthylamide (LLβN) to the biofilm suspension. LLβN is then
977 hydrolyzed by bacteria and produces β-Naphthylamide (βN) whose fluorescence is measured

at 410 nm excitation and 340 nm emission wavelengths. The production rate of βN allows the estimation of bacterial biomass, since there is a linear relationship between both aspects (Batté et al. 2003a).

6. Conclusions

The development of devices to study DW biofilms aims to mimic real DWDS in order to gather results that can be transposed to reality. The use of an appropriate device is an important factor to obtain reproducible and reliable results and should be selected taking into account the goals of the study. While some of the reactors described in this study are mostly used for lab-scale experiments, other reactors are used in real DWDS or under process conditions similar to those found in DWDS. The application of these devices is diverse, going from studies on biofilm formation, monitoring and behavior to studies on biofilm population dynamics and their control from the DWDS. Even if the amount of information on DW biofilms is significant, the dispersal on the experimental process (hydrodynamics, presence/absence of nutrients, presence/absence of disinfectants, type of disinfectants; type of surface material), environmental (temperature, water characteristics) and biological (type of microorganism, single species or mixed species) conditions used do not allow the selection of a best reactor to study DW biofilms. The advantages and limitations should be evaluated *a priori* in order to choose an adequate device to obtain reproducible results that can be transposed to the reality of the DWDS.

Acknowledgements

This work was supported by the Operational Programme for Competitiveness Factors-COMPETE and by FCT-the Portuguese Foundation for Science and Technology through

Project Bioresist-PTDC/EBB-EBI/105085/2008 and the post-doctoral grant SFRH/BPD/81982/2011 (Lúcia C. Simões).

REFERENCES

Abe, Y., Polyakov, P., Skali-Lami, S., Francius, G. 2011. Elasticity and physico-chemical properties during drinking water biofilm formation. Biofouling 27(7), 739-750.

Abe, Y., Skali-Lami, S., Block, J.C., Francius, G. 2012. Cohesiveness and hydrodynamic properties of young drinking water biofilms. Water Res 46(4), 1155-1166.

Altman, S.J., McGrath, L.K., Souza, C.A., Murton, J.K., Camper, A.K. 2009. Integration and decontamination of *Bacillus cereus* in *Pseudomonas fluorescens* biofilms. J Appl Microbiol 107(1), 287-299.

Armbruster, C.R., Forster, T.S., Donlan, R.M., O'Connell, H.A., Shams, A.M., Williams, M.M. 2012. A biofilm model developed to investigate survival and disinfection of *Mycobacterium mucogenicum* in potable water. Biofouling 28(10), 1129-1139.

Bachmann, R.T., Edyvean, R.G.J. 2006. AFM study of the colonisation of stainless steel by *Aquabacterium commune*. Int Biodeter and Biodegr 58(3-4), 112-118.

Batté, M., Koudjonou, B., Laurent, P., Mathieu, L., Coallier, J., Prevost, M. 2003a. Biofilm responses to ageing and to a high phosphate load in a bench-scale drinking water system. Water Res 37(6), 1351-1361.

Batté, M., Mathieu, L., Laurent, P., Prévost, M. 2003b. Influence of phosphate and disinfection on the composition of biofilms produced from drinking water, as measured by fluorescence in situ hybridization. Can J Microbiol 49(12), 741-753.

Bauman, W.J., Nocker, A., Jones, W.L., Camper, A.K. 2009. Retention of a model pathogen in a porous media biofilm. Biofouling 25(3), 229-240.

Beech, I.B., Smith, J.R., Steele, A.A., Penegar, I., Campbell, S. 2002. The use of atomic force microscopy for studying interactions of bacterial biofilms with surfaces. Colloids Surf B 23 (2-3), 231 - 247.

Beech, I.B., Sunner, J. 2004. Biocorrosion: Towards understanding interactions between biofilms and metals. Curr Opin Biotech 15(3), 181-186.

Berry, D., Xi, C., Raskin, L. 2006. Microbial ecology of drinking water distribution systems. Curr Opin Biotech 17(3), 297-302.

Boe-Hasen, R., Albrechtsen, H.J., Arvin, E., Jørgensen, C. 2002. Bulk water phase and biofilm growth in drinking water at low nutrient conditions. Water Res 36(18), 4477-4486.

Boe-Hansen, R., Martiny, A.C., Arvin, E., Albrechtsen, H.J. 2003. Monitoring biofilm formation and activity in drinking water distribution networks under oligotrophic conditions. Water Sci Technol 47(5), 91-97.

Bott, T.R. 1993. Aspects of biofilm formation and destruction. Corrosion Rev 11(1-2), 1-24.

1039 Bridier, A., Meylheuc, T., Briandet, R. 2013. Realistic representation of *Bacillus subtilis*
1040 biofilms architecture using combined microscopy (CLSM, ESEM and FESEM). Micron 48,
1041 65-69.

1042 Butterfield, P.W., Camper, A.K., Ellis, B.D. Jones, W.L. 2002. Chlorination of model
1043 drinking water biofilm: Implications for growth and organic carbon removal. Water Res
1044 36(17), 4391-4405.

1045 Camper, A.K. 2004. Involvement of humic substances in regrowth. Int J Food Microbiol
1046 92(3), 355-364.

1047 Carter, J.T., Rice, E.W., Buchberger, S.G., Lee, Y. 2000. Relationships between levels of
1048 heterotrophic bacteria and water quality parameters in a drinking water distribution system.
1049 Water Res 34(5), 1495-1502.

1050 Chandy, J.P., Angles, M.L. 2001. Determination of nutrients limiting biofilm formation and
1051 the subsequent impact on disinfectant decay. Water Res 35(11), 2677-2682.

1052 Chang, L., Craik, S. 2012. Laboratory simulation of the effect of ozone and monochloramine
1053 on biofilms in drinking water mains. Ozone: Sci Eng 34(4), 243-251.

1054 Chang, Y.C., Le Puil, M., Biggerstaff, J., Randall, A.A., Schulte, A., Taylor, J.S. 2003. Direct
1055 estimation of biofilm density on different pipe material coupons using a specific DNA-probe.
1056 Mol Cel Probes 17(5), 237-243.

1057 Characklis, W.G., Marshall, K.C. 1990. Biofilms. Wiley Series in Ecological andApplied
1058 Microbiology. John Wiley & Sons, USA, 288.

1059 Childs, P.R.N. 2011. Rotaing flow. Elsevier: Butterworth-Heinemann, UK, Chapter 6, 177,
1060 193-203.

1061 Codony, F., Morató, J., Mas, J. 2005. Role of discontinuous chlorination on microbial
1062 production by drinking water biofilms. Water Res 39(9), 1896-1906.

1063 Dailloux, M., Albert, M., Laurain, C., Andolfatto, S., Lozniewski, A., Hartemann, P.,
1064 Mathieu, L. 2003. *Mycobacterium xenopi* and drinking water biofilms. Appl Environ
1065 Microbiol 69(11), 6946-6948.

1066 Deines, P., Sekar, R., Husband, P.S., Boxall, J.B., Osborn, A.M., Biggs, C.A. 2010. A new
1067 coupon design for simultaneous analysis of *in situ* microbial biofilm formation and
1068 community structure in drinking water distribution systems. Appl Microbiol Biotechnol
1069 87(2), 749-756.

1070 Delahaye, E., Levi, Y., Leblon, G., Montiel, A. 2006. A simple system for biofilm potential
1071 monitoring in drinking water. J Basic Microbiol 46(1), 22-27.

1072 Douterelo, I., Sharpe, R.L., Boxall, J.B. 2013. Influence of hydraulic regimes on bacterial
1073 community structure and composition in an experimental drinking water distribution system.
1074 Water Res 47(2), 503-516.

1075 DuBois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith. 1956. Colorimetric method
1076 for determination of sugars and related substances. Anal Chem 28 (3):350-356.

1077 Dykstra, T.S., O'Leary, K.C., Chauret, C., Andrews, R.C., Gagnon, G.A. 2007. Impact of UV
1078 and secondary disinfection on microbial control in a model distribution system. J Environ
1079 Eng Sci 6(2), 147-155.

1080 Emtiazi, F., Schwartz, T., Marten, S.M., Krolla-Sidenstein, P., Obst, U. 2004. Investigation
1081 of natural biofilms formed during the production of drinking water from surface water
1082 embankment filtration. Water Res 38(5), 1197-1206.

1083 Fang, W., Hu, J.Y., On, S.L. 2009. Influence of phosphorus on biofilm formation in model

1084 drinking water distribution systems. J Appl Microbiol 106 (4), 1328-1335.

1085 Fang, W., Hu, J., Ong, S.L. 2010. Effects of phosphorus on biofilm disinfections in model
1086 drinking water distribution systems. J Water Health 8(3), 446-454.

1087 Gagnon, G.A., O'Leary, K.C., Volk, C.J., Chauret, C., Stover, L., Andrews, R.C. 2004.
1088 Comparative analysis of chlorine dioxide, free chlorine and chloramines on bacterial water
1089 quality in model distribution systems. J Environ Eng 130(11), 1269-1279.

1090 Gagnon, G.A., Rand, J.L., O'Leary K, C., Rygel, A.C., Chauret, C., Andrews, R.C. 2005.
1091 Disinfectant efficacy of chlorite and chlorine dioxide in drinking water biofilms. Water Res
1092 39(9), 1809-1817.

1093 Gião, M.S., Azevedo, N.F., Wilks, S.A., Vieira, M.J., Keevil, C.W. 2008. Persistence of
1094 *Helicobacter pylori* in heterotrophic drinking-water biofilms. Appl Environ Microbiol
1095 74(19), 5898-5904.

1096 Gião, M.S., Azevedo, N.F., Wilks, S.A., Vieira, M.J., Keevil, C.W. 2011. Interaction of
1097 *Legionella pneumophila* and *Helicobacter pylori* with bacterial species isolated from
1098 drinking water biofilms. BMC Microbiol 11(57), 1471-2180.

1099 Ginige, M.P., Wylie, J., Plumb, J. 2011. Influence of biofilms on iron and manganese
1100 deposition in drinking water distribution systems. Biofouling 27(2), 151-163.

1101 Goeres, D.M., Loetterle, L.R., Hamilton, M.A., Murga, R., Kirby, D.W., Donlan, R.M. 2005.
1102 Statistical assessment of a laboratory method for growing biofilms. Microbiology 151(3),
1103 757-762.

1104 Goeres, D.M. 2006. Design of model reactor system for evaluating disinfectants against
1105 biofilm bacteria. PhD Dissertation, Montana State University.

1106 Gosselin, F., Madeira, L.M., Juhna, T., Block, J.C. 2013. Drinking water and biofilm
1107 disinfection by Fenton-like reaction. Water Res 47(15), 5631-5638.

1108 Hallam, N.B., West, J.R., Forster, C.F., Simms, J. 2001. The potential for biofilm growth in
1109 water distribution systems. Water Res 35(17), 4063-4071.

1110 Helmi, K., Menard-Szczebara, F., Lénès, D., Jacob, P., Jossent, J., Barbot, C., Delabre, K.
1111 Arnal, C. 2010. Adenovirus, MS2 and PhiX174 interactions with drinking water biofilms
1112 developed on PVC, cement and cast iron. Water Sci Technol 61(12), 3198-3207.

1113 Hosni, A.A., Szabo, J.G., Bishop, P.L. 2011. Efficacy of chlorine dioxide as a disinfectant
1114 for *Bacillus* spores in drinking-water biofilms. J Environ Eng 137(7), 569-574.

1115 Huang, C-T., Peretti, S.W., Bryers, J.D. 1992. Use of flow cell reactors to quantify biofilm
1116 formation kinetics. Biotechnol Tech, 6(3), 193-198.

1117 Jang, H.J., Choi, Y.J., Ka, J.O. 2011. Effects of diverse water pipe materials on bacterial
1118 communities and water quality in the annular reactor. J Microbiol Biotechnol 21(2), 115-123.

1119 Jang, H.J., Choi, Y.J., Ro, H.M., Ka, J.O. 2012. Effects of phosphate addition on biofilm
1120 bacterial communities and water quality in annular reactors equipped with stainless steel and
1121 ductile cast iron pipes. J Microbiol 50(1), 17-28.

1122 Jass, J., Costerton, J.W., Lappin-Scott, H.M. 1995. Assessment of a chemostat-coupled
1123 modified Robbins device to study biofilms. J Ind Microbiol 15(4), 283-289.

1124 Juhna, T., Birzniece, D., Larsson, S., Zulenkovs, D., Sharipo, A., Azevedo, N.F., Ménard-
1125 Szczebara, F., Castagnet, S., Féliers, C., Keevil, C.W. 2007. Detection of *Escherichia coli* in
1126 biofilms from pipe samples and coupons in drinking water distribution networks. Appl
1127 Environ Microbiol 73(22), 7456-7464.

1128 Jungfer, C., Friedrich, F., Varela, J.V., Brandle, K., Gross, H.J., Obst, U., Schwartz, T. 2013.
1129 Drinking water biofilms on cooper and stainless steel exhibit specific molecular responses
1130 towards different disinfection regimes at water works. Biofouling 29(8), 891-907.

1131 Kalmbach, S., Manz, W.,Szewyk, U. 1997. Dynamics of biofilm formation in drinking water:
1132 Phylogenetic affiliation and metabolic potential of single cells assessed by formazan
1133 reduction and in situ hybridization. FEMS MIcrobiol Ecol 22(4), 265-279.

1134 Keinänen-Toivola, M.M., Revetta, R.P., Santo Domingo, J.W. 2006. Identification of active
1135 bacterial communities in a model drinking water biofilm system using 16S rRNA-based
1136 clone libraries. FEMS Microbiol Lett 257(2), 182-188.

1137 Kerr, C.J., Jones, C.R., Hillier, V.F., Robson, G.D., Osborn, K.S., Handley, P.S. 2000.
1138 Statistical evaluation of a newly modified Robbins device using a bioluminescent
1139 pseudomonad to quantify adhesion to plastic. Biofouling 14(4), 267-277.

1140 Långmark, J., Storey, M.V., Ashbolt, N.J., Stenström, T.A. 2005. Accumulation and fate of
1141 microorganisms and microspheres in biofilms formed in a pilot-scale water distribution
1142 system. Appl Environ Microbiol 71(2), 706-712.

1143 LeChevallier, M., Norton, C., Camper, A., Morin, P., Ellis, B., Jones, W., Rompré, A.,
1144 Prevost, M., Coallier, J., Servais, P., Holt, D., Delanowe, A., Colbourn, J. 1998. Microbial
1145 impact of biological filtration. AWWA Res Found, USA, 140-154.

1146 Lehtola, M.J., Miettinen, I.T., Martikainen, P.J. 2002. Biofilm formation in drinking water
1147 affected by low concentrations of phosphorus. Can J Microbiol 48(6), 494-499.

1148 Lehtola, M.J., Torvinen, E., Miettinen, I.T., Keevil, C.W. 2006. Fluorescence in situ
1149 hybridization using peptide nucleic acid probes for rapid detection of Mycobacterium avium
1150 subsp. avium and *Mycobacterium avium* subsp. paratuberculosis in potable-water biofilms.
1151 Appl Environ Microbiol 72(1), 848-853.

1152 Lehtola, M.J., Torvinen, E., Kusnetsov, J., Pitkänen, T., Maunula, L., Von Bonsdorff, C.H.,
1153 Martikainen, P.J., Wilks, S.A., Keevil, C.W., Miettinen, I.T. 2007. Survival of
1154 *Mycobacterium avium*, *Legionella pneumophila*, *Escherichia coli*, and *Calicivirases* in
1155 drinking water-associated biofilms grown under high-shear turbulent flow. Appl Environ
1156 Microbiol 73(9), 2854-2859.

Ling, F., Liu, W.T. 2013. Impact of chloramination on the development of laboratory grown biofilms fed with filter-pretreated groundwater. Microbes Environ 28(1), 50-57.

Liu, G., Verberk, J.Q, Van Dijk, J.C. 2013. Bacteriology of drinking water distribution systems: an integral and multidimensional review. Appl Microbiol Biotechnol 97(21), 9256-9276.

Manuel, C.M., Nunes, O.C., Melo, L.F. 2007. Dynamics of drinking water biofilm in flow/non-flow conditions. Water Res 41(3), 551-562.

Manuel, C.M., Nunes, O.C., Melo, L.F. 2010. Unsteady state flow and stagnation in distribution systems affect the biological stability of drinking water. Biofouling 26(2), 129-139.

Manz, W., Szewzyk, U., Ericsson, P., Amann, R., Schleifer, K.H., Stenstrom, T.A. 1993. *In situ* identification of bacteria in drinking water and adjoining biofilms by hybridization with 16S and 23S rRNA-directed fluorescent oligonucleotide probes. Appl Environ Microbiol 59(7), 2293-2298.

Mathieu, L., Bertrand, I., Abe, Y., Angle, E., Block, J.C., Skali-Lami, S., Francius, G. 2014. Drinking water biofilm cohesiveness changes under chlorination or hydrodynamic stress. Water Res 55, 175-184.

Melo, L.F., Bott, T.R. 1997. Biofouling in water systems. Exp Therm Fluid Sci 14(4), 375-381.

Morato, J., Codony, F., Mas, J. 2005. Utilisation of a packed-bed biofilm reactor for the determination of the potential of biofilm accumulation in water systems. Biofouling 21(3-4), 151-160.

Morin, P., Camper, A.K. 1997. Attachment and fate of carbon fines in simulated drinking water distribution system biofilms. Water Res 31(3), 399-410.

Morrow, J.B., Almeida, J.L., Fitzgerald, L.A., Cole, K.D. 2008. Association and decontamination of *Bacillus s*pores in a simulated drinking water system. Water Res 42(20), 5011-5021.

Murga, R., Forster, T.S., Brown, E., Pruckler, J.M., Fields, B.S., Donlan, R.M. 2001. Role of biofilms in the survival of *Legionella pneumophila* in a model potable-water system. Microbiology 147(11), 3121-3126.

Murphy, H.M., Payne, S.J., Gagnon, G.A. 2008. Sequential UV- and chlorine-based disinfection to mitigate *Escherichia coli* in drinking water biofilms. Water Res 42(8-9), 2083-2092.

Ndiongue, S., Huck, P.M., Slawson, R.M. 2005. Effects of temperature and biodegradable organic matter on control of biofilms by free chlorine in a model drinking water distribution system. Water Res 39(6), 953-964.

Nesic, S., Solvi, G.T., Skjerves, S. 1997. Comparison of rotating cylinder and loop methods for testing CO_2 corrosion inhibitors. Corr Eng Sci Technol 32(4), 269-276.

Nickel, J.C., Ruseska, I., Wright, J.B., Costerton, J.W. 1985. Tobramycin resistance of *Pseudomonas aeruginosa* cells growing as a biofilm on urinary catheter material. Antimicrob Agents Chemother 27(4), 619-624.

O'Toole, G., Kaplan, H.B., Kolter, R. 2000. Biofim formation as microbial development. Annu Rev Microbiol 54(1), 49-79.

Okabe, S., Hirata, K., Watanabe, Y. 1995. Dynamic changes in spatial microbial distribution in mixed-population biofilms: Experimental results and model simulation. Water Sci Technol 32(8), 67-74.

Ollos, P.J., Huck, P.M., Slawson, R.M. 2003. Factors affecting biofilm accumulation in model distribution systems. J Amer Water Works Assoc 95(1), 87-97.

Palmer, R.J., Sterneberg, C. 1999. Modern microscopy in biofilm research: confocal microscopy and other approaches. Curr Opin Biotechnol 10 (3), 263 - 268.

Paris, T., Skali-Lami, S., Block, J.C. 2007. Effect of wall shear rate on biofilm deposition and grazing in drinking water flow chambers. Biotechnol Bioeng 97(6), 1550-1561.

Paris, T., Skali-Lami, S., Block, J.C. 2009. Probing young drinking water biofilms with hard and soft particles. Water Res 43(1), 117-126.

Park, S.K., Hu, J.Y. 2010. Assessment of the extent of bacterial growth in reverse osmosis system for improving drinking water quality. J Environ Sci Health 45(8), 968-977.

Park, S.-K., Choi, J.-H., Hu, J.Y. 2012. Assessing bacterial growth potential in a model distribution system receiving nanofiltration membrane treated water. Desalination 296(0), 7-15.

Pedersen, K. 1982. Method for studying microbial biofilms in flowing-water systems. Appl Environ Microbiol 43(1), 6-13.

Pelleïeux, S., Bertrand, I., Skali-Lami, S., Mathieu, L., Francius, G., Gantzer, C. 2012. Accumulation of MS2, GA, and Qβ phages on high density polyethylene (HDPE) and drinking water biofilms under flow/non-flow conditions. Water Res 46(19), 6574-6584.

Percival, S.L., Knapp, J.S., Edyvean, R.G.J., Wales, D.S. 1998. Biofilms, mains water and stainless steel. Water Res 32(7), 2187-2220.

Percival, S.L., Knapp, J.S., Wales, D.S., Edyvean, R.G.J. 1999. The effect of turbulent flow and surface roughness on biofilm formation in drinking water. J Ind Microbiol Bitechnol 22(3), 152 – 159.

Pérez, J.A.S., Porcel, E.M.R, López, J.L.C, Sevilla, J.M.F, Chisti, Y. 2006. Shear rate in stirred tank and bubble column bioreactors. Chem Eng J, 124(1-3), 1-5.

Pintar, K.D.M., Slawson, R.M. 2003. Effect of temperature and disinfection strategies on ammonia-oxidizing bacteria in a bench-scale drinking water distribution system. Water Res 37(8), 1805-1817.

Prévost, M., Rompré, A., Coallier, J., Servais, P., Laurent, P., Clément, B., Lafrance, P. 1998. Suspended bacteria biomass and activity in full-scale drinking water distribution system: Impact of water treatment. Water Res 32(5), 1393-1406.

Rand, J.L., Hofmann, R., Alam, M.Z.B., Chauret, C., Cantwell, R., Andrews, R.C., Gagnon, G.A. 2007. A field study evaluation for mitigating biofouling with chlorine dioxide or chlorine integrated with UV disinfection. Water Res 41(9), 1939-1948.

Rickard, A.H., McBain, A.J., Stead, A.T., Gilbert, P. 2004. Shear rate moderates community diversity in freshwater biofilms. Appl Environ Microbiol 70(12), 7426-7435.

Rochex, A., Godon, J.J., Bernet, N., Escudie, R. 2008. Role of shear stress on composition, diversity and dynamics of biofilm bacterial communities. Water Res 42(20), 4915-4922.

Rubulis, J., Juhna, T. 2007. Evaluating the potential of biofilm control in water supply systems by removal of phosphorus from drinking water. Water Sci Technol 55(8-9), 211-217.

Sammon, N.B., Harrower, K.M., Fabbro, L.D., Reed, R.H. 2011. Three potential sources of microfungi in a treated municipal water supply system in sub-tropical Australia. Int J Environ Res Public Health 8(3), 713-732.

Schaule, G., Moschnitschka, D., Schulte, S., Tamachkiarow, A., Flemming, H.C. 2007. Biofilm growth in response to various concentrations of biodegradable material in drinking water. Water Sci Technol 55(8-9), 191-195.

Schlichting, H. 1955. Boundary-layer theory, Mc Graw - Hill, New York, 535.

Sharp, R.R., Camper, A.K., Crippen, J.J., Schneider, O.D., Leggiero, S. 2001. Evaluation of drinking water biostability using biofilm methods. J Environ Eng 127(5), 403-410.

Sierack, M. E., Cucci, T. L., Nicinski, J. 1999. Flow cytometric analysis of 5-cyano-2,3-ditolyl tetrazolium chloride activity of marine bacterioplankton in dilution cultures. App Environ Microbiol 65(6), 2409-2417.

Silvestry-Rodriguez, N., Bright, K.R., Slack, D.C., Uhlmann, D.R., Gerba, C.P. 2008. Silver as a residual disinfectant to prevent biofilm formation in water distribution systems. Appl Environ Microbiol 74(5), 1639-1641.

Simões, L.C., Azevedo, N., Pacheco, A., Keevil, C.W., Vieira, M.J. 2006. Drinking water biofilm assessment of total and culturable bacteria under different operating conditions. Biofouling 22(2), 91-99.

Simões, L.C., Simões, M., Oliveira, R., Vieira, M.J. 2007. Potential of the adhesion of bacteria isolated from drinking water to materials. J Basic Microbiol 47(2), 174-183.

Simões, L.C., Simões, M., Vieira, M.J. 2010a. Adhesion and biofilm formation on polystyrene by drinking water-isolated bacteria. Antonie van Leeuwenhoek, Int J Gen Mol Microbiol 98(3), 317-329.

Simões, L.C., Simões, M., Vieira, M.J. 2010b. Influence of the diversity of bacterial isolates from drinking water on resistance of biofilms to disinfection. Appl Environ Microbiol 76(19), 6673-6679.

Simões, L.C., Simões, M., Vieira, M.J. 2011. The effects of metabolite molecules produced by drinking water-isolated bacteria on their single and multispecies biofilms. Biofouling 27(7), 685-699.

Simões, L.C., Simões, M., Vieira, M.J. 2012. A comparative study of drinking water biofilm monitoring with flow cell and Propella™ bioreactors. Water Sci Technol 12(3), 334-342.

Simões, L.C., Simões, M. 2013. Biofilms in drinking water: Problems and solutions. RSC Advances 3(8), 2520-2533.

1277 Siqueira, V.M., Oliveira, H.M.B., Santos, C., Paterson, R.R.M., Gusmão, N.B., Lima, N.
1278 2011. Filamentous fungi in drinking water, particularly in relation to biofilm formation. Int J
1279 Environ Res Public Health 8(2), 456-469.

1280 Siqueira, V.M., Oliveira, H.M.B., Santos, C., Paterson, R.R.M., Gusmão, N.B., Lima, N.
1281 2013. Biofilms from a Brazilian water distribution system include filamentous fungi. Can J
1282 Microbiol 59(3), 183-188.

1283 Sly, L.I., Hodgkinson, M.C., Arunpairojana, V. 1990. Deposition of manganese in a drinking
1284 water distribution system. Appl Environ Microbiol 56(3), 628-639.

1285 Stoodley, P., Dodds, I., Boyle, J.D., Lappin-Scott, H.M. 1999. Influence of hydrodynamics
1286 and nutrients on biofilm structure. J App Microbiol Symposium Supp 85(28), 19S-28S.

1287 Szabo, J.G., Rice, E.W., Bishop, P.L. 2007. Persistence and decontamination of *Bacillus*
1288 *atrophaeus subsp. globigii* spores on corroded iron in a model drinking water system. Appl
1289 Environ Microbiol 73(8), 2451-2457.

1290 Szabo, J.G., Impellitteri, C.A., Govindaswamy, S., Hall, J.S. 2009. Persistence and
1291 decontamination of surrogate radioisotopes in a model drinking water distribution system.
1292 Water Res 43(20), 5005-5014.

1293 Teng, F., Guan, Y.T., Zhu, W.P. 2008. Effect of biofilm on cast iron pipe corrosion in
1294 drinking water distribution system: Corrosion scales characterization and microbial
1295 community structure investigation. Corrosion Sci 50(10), 2816-2823.

1296 Teodósio, J.S., Simões, M., Alves, M.A., Melo, L.F., Mergulhão, F.J. 2012. Setup and
1297 validation of flow cell systems for biofouling simulation in industrial settings. The Scientific
1298 World Journal 2012(2012), 180-187.

1299 Torvinen, E., Lehtola, M.J., Martikainen, P.J., Miettinen, I.T. 2007. Survival of
1300 *Mycobacterium avium* in drinking water biofilms as affected by water flow velocity,
1301 availability of phosphorus, and temperature. Appl Environ Microbiol 73(19), 6201-6207.

1302 van der Kooij, D., Veenendaal, H.R., Baars-Lorist, C., van der Klift, D.W., Drost, Y.C. 1995.
1303 Biofilm formation on surfaces of glass and Teflon exposed to treated water. Water Res 29(7),
1304 1655-1662.

1305 van der Kooij, D. 1999. Potential for biofilm development in drinking water distribution
1306 systems. J App Microbiol Symposium Supp 85(28), 39S-44S.

1307 Volk, C.J., LeChevallier, M.W. 1999. Impacts of the reduction of nutrient levels on bacterial
1308 water quality in distribution systems. Appl Environ Microbiol 65(11), 4957-4966.

1309 Wadhawan, T., McEvoy, J., Prüβ, B. M., Khan, E. 2010. Assessing tetrazolium and ATP
1310 assays for rapid in situ viability quantification of bacterial cells entrapped in hydrogel beads.
1311 Enzyme and Microbial Technol 47(4), 166 – 173.

1312 WHO 2011. Guidelines for drinking water quality 4th edition World Health Organization.

1313 Willcock, L., Gilbert, P., Holah, J., Wirtanen, G., Allison, D.G. 2000. A new technique for
1314 the performance evaluation of clean-in-place disinfection of biofilms. J Ind Microbiol
1315 Biotech 25(5), 235-241.

Wingender, J., Neu, T.R., Flemming, H.C. 1999. Microbial extracellular polymeric substances: characterization, structures and function. Springer – Verlag, Berlin Heidelber Chapter 3.

Wingender, J., Flemming, H.C. 2004. Contamination potential of drinking water distribution network biofilms. Water Sci Technol 49(11-12), 277-286.

Yu, W., Doods, W.K., Banks, M.K., Skalsky, J., Strauss, E.A. 1995. Optimal staining and sample storage time for direct microscopic enumeration of total and active bacteria in soil with two fluorescent dyes. App Environ Microbiol 6(9), 3367 - 3372.

Yu, J., Kim, D., Lee, T. 2010. Microbial diversity in biofilms on water distribution pipes of different materials. Water Sci Technol 61(1), 163-171.

Zhang, Y., Zhou, L., Zeng, G., Deng, H., Li, G. 2010. Impact of total organic carbon and chlorine to ammonia ratio on nitrification in a bench-scale drinking water distribution system. Frontiers Environ Sci Eng China 4(4), 430-437.

Zhou, L.L., Zhang, Y.J., Li, G.B. 2009. Effect of pipe material and low level disinfectants on biofilm development in a simulated drinking water distribution system. J Zhejiang University: Sci A 10(5), 725-731.

Figures and Tables

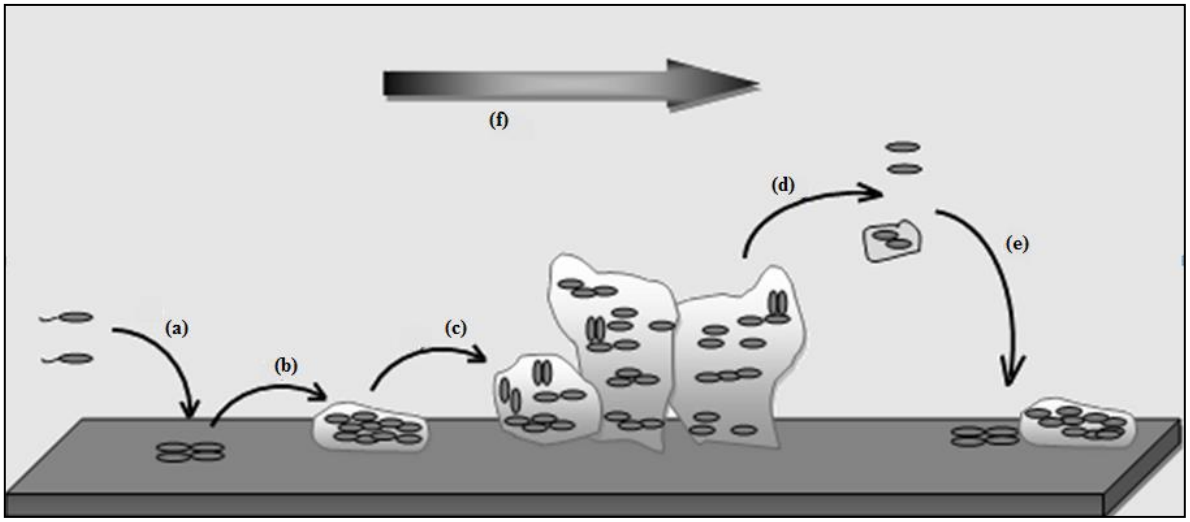


Fig. 1 - Biofilm formation, detachment and recolonization in DWDS. (a) Attachment, (b) initiation, formation of colonies, starting of EPS production, (c) biofilm maturation, (d) biofilm dissolution, (e) biofilm recolonization of DWDS pipes influenced by the water flow (f). Based on Codony et al. (2005).

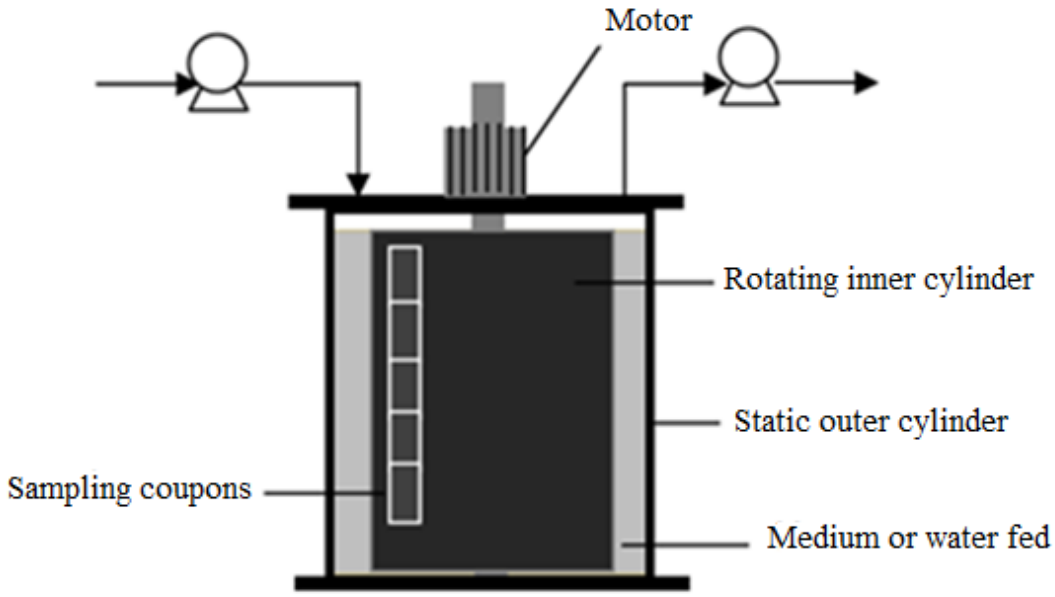


Fig. 2 - Annular reactor, cross-sectional view. The operational mode (batch or continuous) can be controlled by the pumps; the shear stress is controlled by the rotation of the inner cylinder.

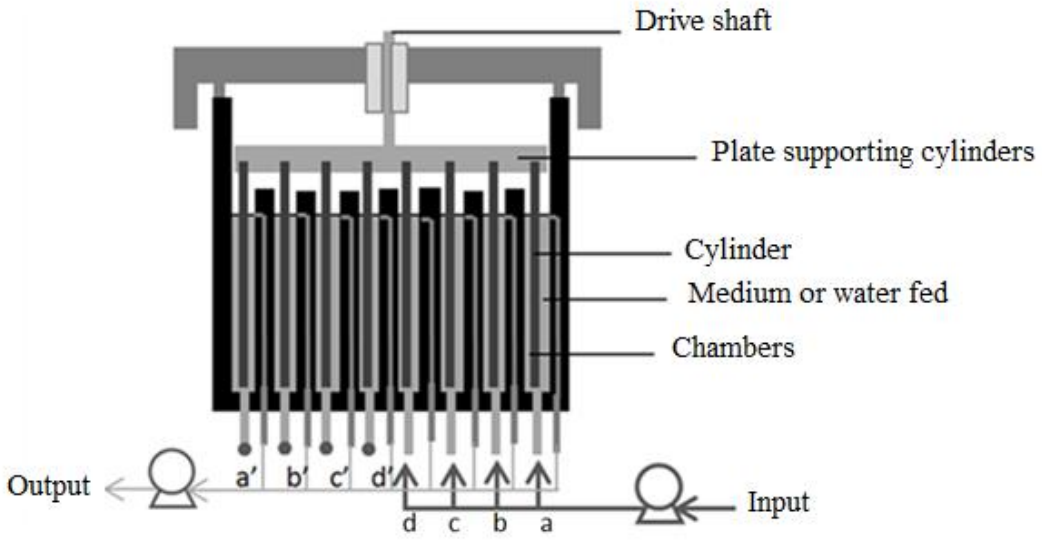


Fig. 3 - Cross- sectional view of the concentric cylinder reactor (CCR), four rotating cylinders interlocked within the four collecting stationary cylinder chambers. a,b,c,d are the inlet ports to fed the medium or water corresponding to the same chambers; a',b',c',d' are the sampling ports in each chamber.

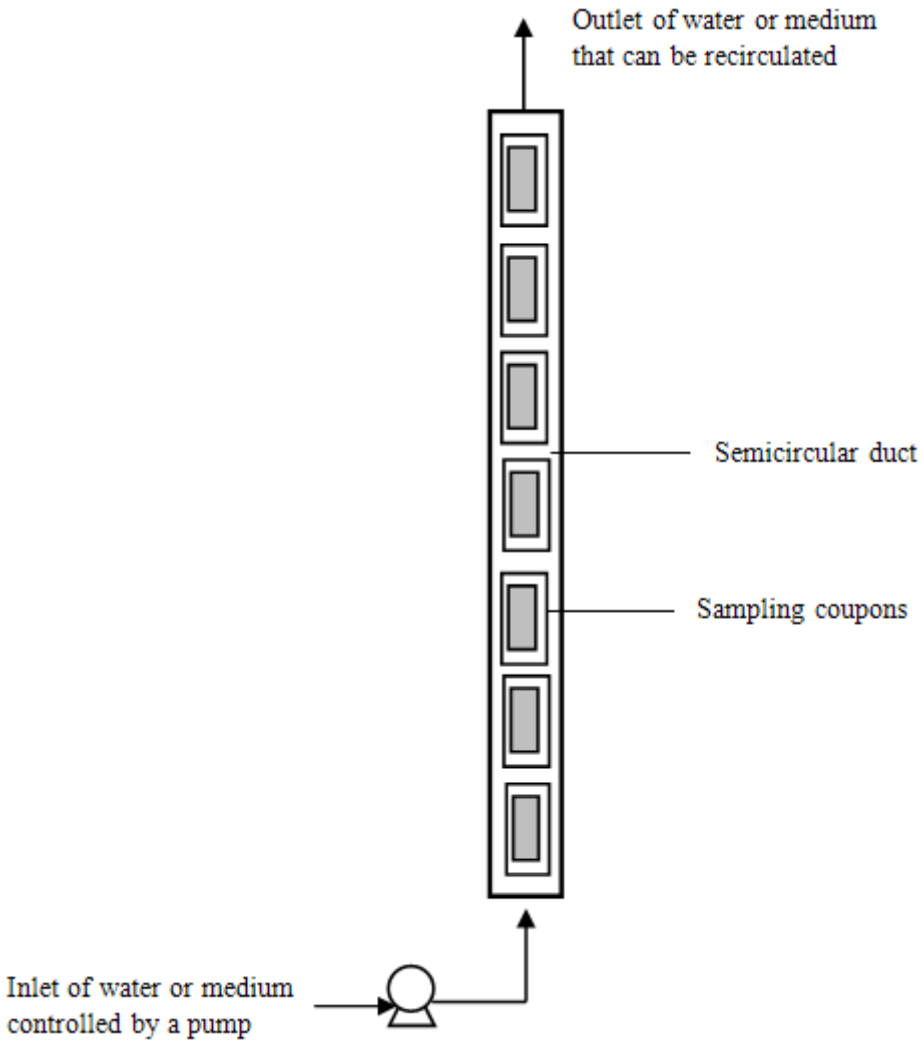


Fig. 4 - Scheme of the flow cell system. The fed can be provided from tap or from reservoirs, the biofilm is formed on the removable coupons and the flow is controlled by external pumps.

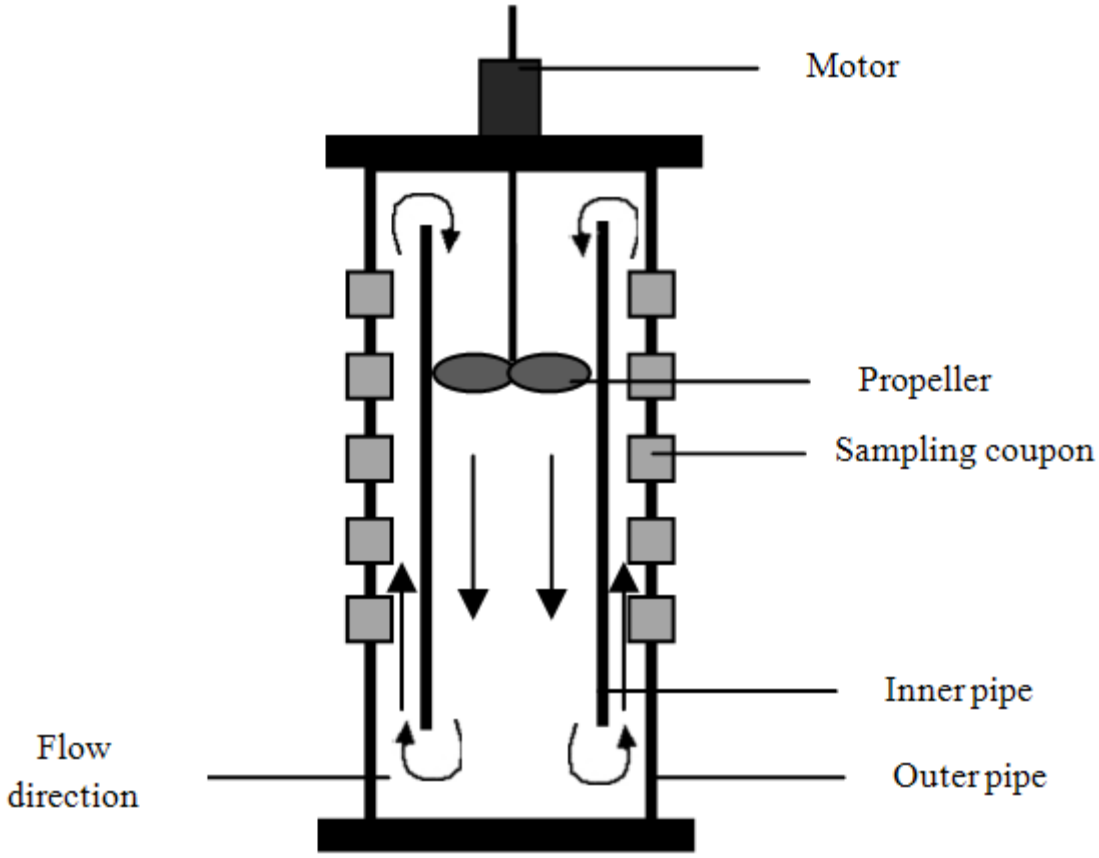


Fig. 5 - Propella® reactor, cross-sectional view. Flow direction is represented by the arrows.

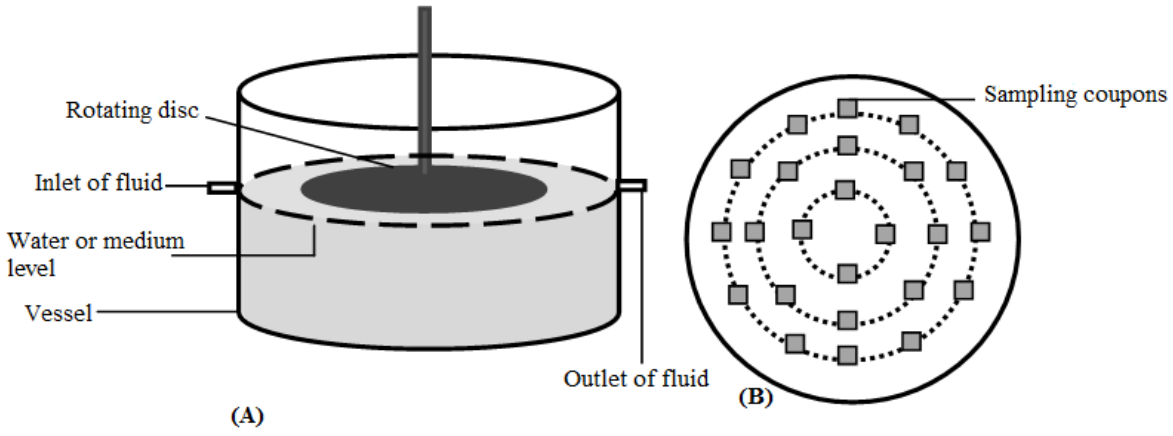


Fig. 6 – (A) Rotating disc reactor (RDR), the inlet and outlet of fluid can be controlled with a pump. (B) Scheme of the disposal of removable coupons in the disc.

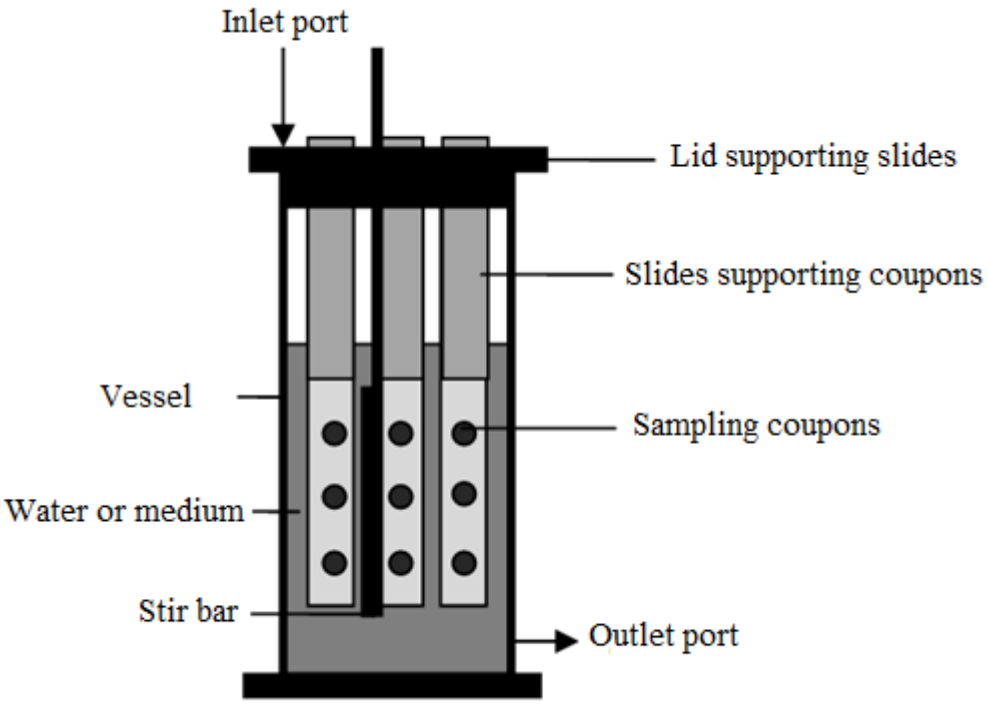


Fig. 7 – Center for disease control (CDC) biofilm reactor, cross-sectional view. The flow is controlled by external pumps.

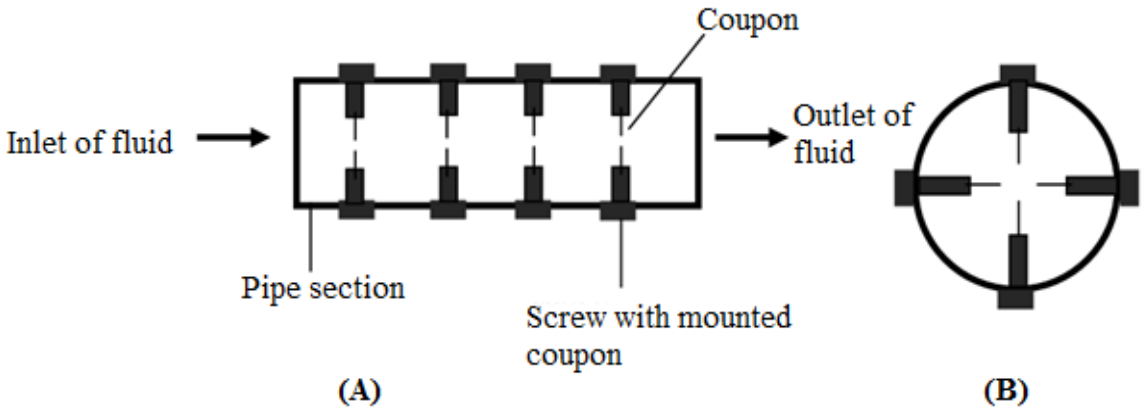


Fig. 8 – Robbins device. (A) Longitudinal section; (B) Cross-sectional view.

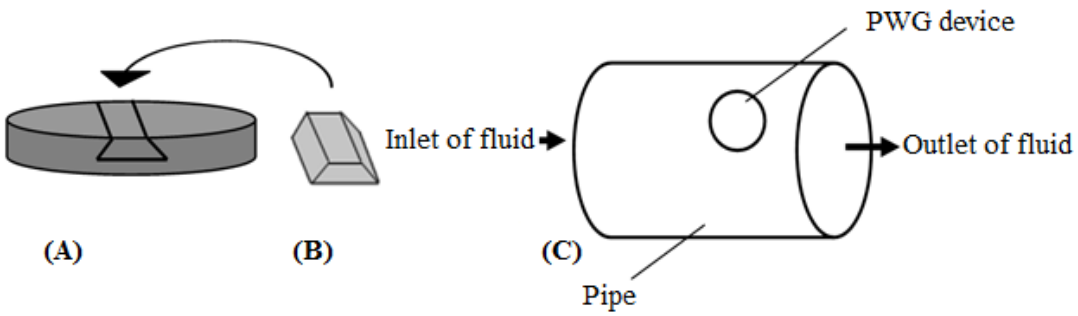
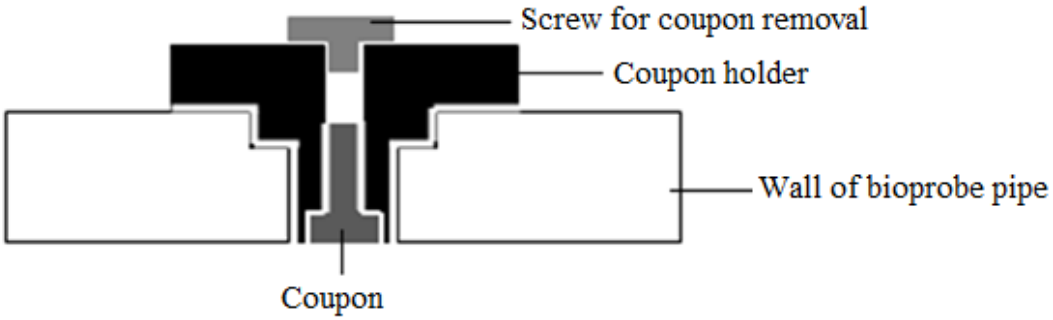


Fig. 9 – Pennine water group (PWG) coupon. (A) The outer coupon, where is located the insert; (B) Insert; (C) Pipe with the appropriate hole to locate the outer coupon with the insert fixed with a gasket.



1384

1385 **Fig. 10 - Scheme of the bioprobe monitor with a coupon insertion in the pipe.**

1386

1387

Table 1- Fluid dynamic equations for DWDS model reactors.

Reactor	Equations	Assumptions/observations	References
Annular reactor	$Re = \frac{N.D_h^2.\rho}{\mu}$; $D_h = D_o - D_i$ $f = \frac{0.0791}{Re^{0.25}}$ $\gamma = \frac{f\rho v^2}{2}$	- Gross simplification - The expressions used are common to closed pipe flow	Altman et al. (2009)
CCR	$Re = \frac{N.D_h^2.\rho}{\mu}$; $D_h = D_o - D_i$ $f = 0.158Re_A^{-0.3}$ $\gamma = \frac{f\rho v^2}{2}$	- Fanning factor is adjusted to rotating cylinders (Nesic et al., 1997) but it still is a gross approach	-
Flow cell reactor; *In situ* devices	$Re = \frac{\rho.v.D_h}{\mu}$; $D_h = \frac{\pi.D}{2+\pi}$ to a semicircular duct $D_h = \frac{2ab}{a+b}$ to a rectangular duct $f = \frac{0.0791}{Re^{0.25}}$ $\gamma = \frac{f\rho v^2}{2}$	-Flow cell: It is used the expression of Fanning factor from circular pipes; - The flow is not changed by the coupons	Teodósio et al. (2012)
Propella® reactor	$Re = \frac{\rho.v.D_h}{\mu}$; $D_h = D_o - D_i$ $f = \frac{0.0791}{Re^{0.25}}$ $\gamma = \frac{f\rho v^2}{2}$	- The flow was not changed by the coupons	
RDR	$\gamma = 0.729r\sqrt{\frac{N^3}{\delta}}$	- From Navier–Stokes equations as described Schlichting (1955)	Pelleïeux et al. (2012)
CDC reactor	$Re = \frac{N.\alpha.R_o^2.\rho}{\mu}$ $Re_{trans.} = \frac{41.3}{(1-\alpha)^{1.5}}$ $f_{turb.} = \frac{0.0791}{Re^{0.25}}$ $f_{lam.} = \frac{16}{Re}$ $\gamma = \frac{f.\rho.N^2.R_i.R_o}{2}$	- Reactor is modeled by two concentric cylinders - Reynolds equation described by Characklis and Marshall (1990) to concentric cylinders.	Goeres (2006)

a and b - dimensions of the rectangular flow section; D - diameter of the semicircular flow section; D_h - hydraulic diameter; D_o - outer diameter; D_i - inner diameter; f - Fanning friction factor; N - rotating speed; R_o - outer radius; R_i - inner radius; v - fluid velocity; α - ratio of inner to outer cylinder; ρ - fluid density; μ - dynamic viscosity; δ – kinematic viscosity; γ - shear stress.

Table 2 - Main advantages and limitations of some of the presented devices.

Reactors	Advantages	Limitations
Annular reactor	Allows the study of different materials at the same time; interesting to assess the role of hydrodynamic conditions on biofilms; high surface area; easy sampling process; shear stress control independent from the fluid flow	The coupons can change the flow patterns; non-ideal mixing; non-uniform biofilm formation
CCR	Interesting to assess the role of hydrodynamic conditions on biofilms; allows testing different shear stress conditions at the same time; allows periodical sampling	Only one surface material can be tested *per* experiment; lack of sufficient sampling surface area; difficult sampling process
Flow cell reactor	Flow conditions similar to DWDS; independent sampling at the desired time without changing or stopping the flow; allows the study of different materials at the same time; easy to control environmental conditions	Flow changed by the coupons; biofilms are formed on a flat surface; lack of sufficient sampling surface area
Propella®	Easy control of the flow conditions; residence time controlled independently from the flowing process; flow conditions very similar to DWDS; allows the simultaneous study of different materials; allows periodical sampling	Changes in the flow caused by coupons; lack of sufficient sampling surface area
RDR	Possibility to study different materials; easy to control of operational conditions; allows testing different shear stresses simultaneously	The flow changes in the boundaries of the coupons; the biofilm is formed on a flat surface; lack of sufficient sampling surface area
CDC biofilm reactor	Allows the study of different materials simultaneously; easy control of hydrodynamic conditions	The surface where biofilms are formed is flat; difficult control of the shear stress; changes of the flow pattern in the boundaries of the coupons; lack of sufficient sampling surface area
Microtiter plates	Needs small space; high-throughput analysis, easy to control environmental conditions; non-invasive analysis of cell adhesion and biofilm formation	Low similarity to DWDS; batch system; unable to study high shear stress conditions; volume limitations
Robbins device	Can be applied to real DWDS with operational conditions very similar to reality; allows the study of different materials simultaneously	The flow characteristics are changed with the presence of the coupons; the operational conditions cannot be effectively controlled when used in real DWDS ; lack of sufficient sampling surface area
MRD	Can be applied to real DWDS with operational conditions very similar to the reality; minimizes the changes in flow in the boundaries of coupons; allows the study of different materials simultaneously	Limitations in the control of operational conditions; lack of sufficient sampling surface area
PWG coupon	Useful to be used at pilot-scale DWDS; do not change the flow conditions, curved structure as the DWDS pipes; lack of sufficient sampling surface area; allows the study of different materials	Limitations in the control of operational conditions; lack of sufficient sampling surface area
Bioprobe monitor	Allows to assess biofilm development *in situ*; changes in water flow are minimized; allows the study of different materials	Limitation in the control of operational conditions; limited available information; lack of sufficient sampling surface area

Table 3 - Overview of DW biofilm studies the main operational conditions and microorganisms used.

Reactor	Factors				Disinfectants	Microorganisms	References
	Material	Hydrodynamics	Temperature	Nutrients			
Annular reactor	Mild steel	Rotation speed: 60 rpm	10 ºC	-	Chlorine (1.3 mg.L^{-1})	Tap water microorganisms	Volk and Le Chevallier (1999)
Annular reactor	Polycarbonate	Residence time: 4.3 h; Flow rate: 3 mL.min^{-1}	25 ºC	Potassium, phosphate and sodium acetate	Chloramine (0.70 to 1.4 mg.L^{-1})	Tap water microorganisms	Chandy and Angles (2001)
Annular reactor	Polycarbonate	Rotation speed: 40 rpm	-	Carbon stock solution (0.235 mg.L^{-1}); phosphate addition (0.5 mg P. L^{-1})	Chlorine and monochlorine (0.6 to 0.9 mg. L^{-1})	Tap water microorganisms	Batté et al. (2003b)
Annular reactor	PVC	Shear stress: 0.25 N/m^2	6, 12 and 22 ºC	Sodium acetate, sodium nitrate and potassium di-hydrogen phosphate	Chloramine (0.2-0.6 mg.L^{-1} and 0.05-0.1 mg.L^{-1}), chlorine (residual concentration)	Ammonia-oxidizing bacteria	Pintar and Slawson (2003)
Annular reactor	Cast iron and polycarbonate	Shear stress: 0.25 N.m^{-2}	20 ºC	-	Chlorite (0.1 and 0.25 mg.L^{-1}) and chlorine dioxide (0.25 and 0.5 mg. L^{-1})	Tap water microorganisms	Gagnon et al. (2004)
Annular reactor	Cast iron and polycarbonate	Shear stress: 0.25 N.m^{-2}	20 ºC	Nitrate, phosphate and biodegradable organic carbon	Free chlorine (0.5 to 1.0 mg. L^{-1}), chlorine dioxide (0.25-0.5 mg.L^{-1}) and chloramines (1 to 2 mg.L^{-1})	Tap water microorganisms	Gagnon et al. (2005)
Annular reactor	PVC	Rotation speed: 92 rpm	6, 12 and 18 ºC	Carbon (0 and 250 µg C/L)	Chlorine (0.05 to 0.23 mg. L^{-1})	Tap water microorganisms	Ndiongue et al. (2005)

Reactor	Factors				Disinfectants	Microorganisms	References
	Material	Hydrodynamics	Temperature	Nutrients			
Annular reactor	SS and medium-density polyethylene (MDPE)	Rotation speed: 150 rpm	15 ºC	-	-	*Aquabacterium commune*	Bachmann and Edyvean (2006)
Annular reactor	Polycarbonate	Shear stress: 0.25 $N.m^{-2}$	20 ºC	Ethyl alcohol, propionaldehyde, oxalate, pyruvate, and acetate	UV radiation (45 $mJ.cm^{-2}$), free chlorine (0.5 and 1.0 $mg.L^{-1}$), chlorine dioxide (0.25 and 0.5 $mg.L^{-1}$) and monochloramine (1.0 and 2.0 $mg.L^{-1}$)	Tap water microorganisms	Dykstra et al. (2007)
Annular reactor	Polycarbonate	Shear stress: 0.68 $N.m^{-2}$	24 ºC	-	UV radiation, chlorine (0.20 $mg.L^{-1}$) and chlorine dioxide	Tap water microorganisms	Rand et al. (2007)
Annular reactor	Polycarbonate	Rotation speed: 100 rpm	21-23 ºC	-	Chlorine (0.6 to 1.0 $mg.L^{-1}$)	*Klebsiella pneumoniae*	Szabo et al. (2007)
Annular reactor	Teflon	-	-	1-3 $mg.L^{-1}$ organic carbon	-	Tap water microorganisms	Schaule et al. (2007)
Annular reactor	Polycarbonate	Re number: 217	10 ºC	-	-	*Cryptosporidium parvum, Giardia lamblia, Vaccinal Poliovirus Type 1*, and *Bacteriophages φX174* and *MS2*	Helmi et al. (2010)
Annular reactor	Polycarbonate and cast iron	Shear stress: 0.25 $N.m^{-2}$	20 ºC	-	UV radiation (16 $mJ.cm^{-2}$), chlorine (0.2 and 1.0 $mg.L^{-1}$), chlorine dioxide (0.2 and 1.0 $mg.L^{-1}$) and monochloramine (1.0 and 2.0 $mg.L^{-1}$)	*Escherichia coli*	Murphy et al. (2008)

Reactor	Factors				Disinfectants	Microorganisms	References
	Material	Hydrodynamics	Temperature	Nutrients			
Annular reactor	SS and Cu	Residence time: 53 min	Room temperature	-	Chlorine (0.6 $mg.L^{-1}$) and chloramines (0.60-0.75 $mg.L^{-1}$)	Tap water microorganisms	Zhou et al. (2009)
Annular reactor	Polycarbonate	Rotation speed: 133 rpm; Retention time: 3 h; velocity: 0.3 $m.s^{-1}$	-	Sodium acetate (200µg $C.mL^{-1}$) and di-hydrogen phosphate (300µg $P.mL^{-1}$)	Free chlorine (0.2-2 $mg.L^{-1}$) and monochloramine (1-4 $mg.L^{-1}$)	Tap water microorganisms	Fang et al. (2010)
Annular reactor	SS	-	20 ºC	-	Chloramine (0.09-0.16 and 0.01-0.06 $mg.L^{-1}$)	Heterotrophic bacteria and ammonia oxidizing bacteria from tap water	Zhang et al. (2010)
Annular reactor	Polycarbonate	-	-	-	Chlorine dioxide (5, 10, 15 and 25 mg. L^{-1})	*Bacillus globigii*	Hosni et al. (2011)
Annular reactor	Steel, SS, Cu and PVC	Shear stress: 0.24 N/m^2	-	-	-	Tap water microorganisms	Jang et al. (2011)
Annular reactor	Cement	-	20 ºC	-	Ozone, monochloramine (2 to 2.5 $mg.L^{-1}$)	Tap water microorganisms	Chang and Craik (2012)
CCR	SS	Fluid velocity: 0.26, 0.19, 0.16 and 0.12 $m.s^{-1}$.	-	-	-	Microorganisms from untreated potable water	Rickard et al. (2004)
Flow cell system	PVC and SS	Re number: 2000 and 11000	20 ºC	Carbon (0.5 $mg.L^{-1}$), nitrogen (0.1 $mg.L^{-1}$) and phosphorus (0.01 $mg.L^{-1}$)	-	Tap water microorganisms	Simões et al. (2006)
Flow cell system	PVC	Re number: 4900 and 810	20 ºC	-	-	Tap water microorganisms	Manuel et al. (2010)
Flow cell reactor and Propella® reactor	PVC, cross linked polyethylene (PEX), HDPE and PP (Polypropylene)	Shear stress: 0.80 and 1.91 Pa	15.9 ºC	-	-	Tap water microorganisms	Manuel et al. (2007)

Reactor	Factors				Disinfectants	Microorganisms	References
	Material	Hydrodynamics	Temperature	Nutrients			
Flow cell system and Propella® reactor	PVC and SS 316	Re number: 2000 and 11 000	20 ºC	-	-	Tap water microorganisms	Simões et al. (2012)
Propella® reactor	PVC	-	-	-	-	*Mycobacterium avium subsp. avium* and *Mycobacterium avium subsp. paratuberculosis*	Lehtola et al. (2006)
Propella® reactor	PVC	Re number: 15000	15 ºC	-	-	*Mycobacterium avium*	Lehtola et al. (2007)
Propella® reactor	PVC	Flow rate: 0.25 $m.s^{-1}$	-	-	-	Tap water microorganisms	Rubulis and Juhna (2007)
Propella® reactor	PVC	Flow velocity: 0.10; 0.24 $m.s^{-1}$	7 and 20 ºC	Phosphorus (4.2, 13.8 µg/L)	Chlorine (0.17 $mg.L^{-1}$)	*Mycobacterium avium*	Torvinen et al. (2007)
Propella® Reactor	PVC and SS 316	Water velocity: 0.13 $m.s^{-1}$; retention time: 12 h	20 ºC	-	Fenton reaction (iron particles at 10^{-1}; 10^{-2}; 5 x 10^{-2} and 10^{-3} M Fe, hydrogen peroxide at $1.5x10^{-2}$ M)	Tap water microorganisms	Gosselin et al. (2013)
RDR	SS	-	30 ºC	Yeast extract, proteose peptone, casamino acids, dextrose (0.5 $g.L^{-1}$) sodium pyruvate and dibasic potassium phosphate (0.03 $g.L^{-1}$), magnesium phosphate (0.005 $g.L^{-1}$)	-	*Legionella pneumophila* and *Hartmannella vermiformis*	Murga et al. (2001)
RDR	Glass	Shear stress: 0.12 Pa	21 ºC	-	-	Tap water microorganisms	Abe et al. (2011)
RDR	HDPE	Shear rate:450-1640 s^{-1}	20 ºC	-	-	MS2, GA and Qβ phages replicated using *E. coli*	Pelleieux et al. (2012)

Reactor	Factors				Disinfectants	Microorganisms	References
	Material	Hydrodynamics	Temperature	Nutrients			
CDC reactor and Pipe loop reactor	PVC and Cu	Flow rate: 1 mL. min^{-1} (pipe loop reactor); 0.3 mL. min^{-1}	-	Humic acids	Free chlorine (10 and 103 $mg.L^{-1}$), monochloramine (13, 49 and 99 $mg.L^{-1}$)	*Bacillus* spores	Morrow et al. (2008)
CDC reactor	PVC	Rotation speed: 50 rpm	25-29 ºC	-	-	Tap water microorganisms	Park and Hu (2010)
CDC reactor	PVC and SS	-	-	-	Monochloramine (1 or 2 mg. L^{-1})	*Sphingomonas paucimobilis, Methylobacterium* sp., *Delftia acidovorans*, and *Mycobacterium mucogenicum*	Armbruster et al. (2012)
CDC reactor	PVC	Shear stress: 0.01 N/m^2	25-29 ºC	-	-	Tap water microorganisms	Park et al. (2012)
Microtiter plates	Polystyrene	-	23 ºC	-	-	*Acinetobacter calcoaceticus, Burkholderia cepacia, Methylobacterium sp., Mycobacterium mucogenicum, Sphingomonas capsulata and Staphylococcus sp.* (DW isolated-bacteria)	Simões et al. (2010a)
Microtiter plates	Polystyrene	-	23 ºC	-	Sodium hypochlorite (0.1, 0.5; 1 and 10 $mg.L^{-1}$)	*Acinetobacter calcoaceticus, Burkholderia cepacia, Methylobacteriumsp., Mycobacterium mucogenicum, Sphingomonas capsulata* and *Staphylococcus sp.* (DW isolated-bacteria)	Simões et al. (2010b)
Glass ring column	Glass	Hydraulic retention time: 0.5 d	25 ºC	Acetate (0.5 $mg.L^{-1}$)	Chlorine (3.0 $mg.L^{-1}$)	Tap water microorganisms, namely *Pseudomonas fluorescens* and *Spirillum species*	Ginige et al. (2011)

Reactor	Factors				Disinfectants	Microorganisms	References
	Material	Hydrodynamics	Temperature	Nutrients			
Robbins device	Polyethylene	Water velocity: 0.5 $m.s^{-1}$	-	-	Chlorine (0.08 to 0.73 $mg.L^{-1}$), chlorine dioxide (<0.01 to 0.27 $mg.L^{-1}$)	Tap water microorganisms	Sly et al. (1990)
MRD	Glass and Polyethylene	Flow rate: 0.6 $L.h^{-1}$	12.2 ºC	-	-	Tap water microorganisms	Kalmbach et al. (1997)
MRD	PVC and SS	-	24 ºC	Humic acid (0.5 $mg.L^{-1)}$	Silver nitrate (0.1 $mg.L^{-1}$)	Tap water microorganisms	Silvestry-Rodriguez et al. (2008)
PWG coupon	High-performance polyethylene (HPPE)	Shear stress: 0.03 $N.m^{-2}$	25 ºC	-	-	Tap water microorganisms	Deines et al. (2010)
PWG coupon	HDPE	Growth conditions (0.2 to 0.5 $L.s^{-1}$, 0.4 $L.s^{-1}$ and 0.2 to 0.8 $L.s^{-1}$); flushing conditions (0.2 to 3 $N.m^{2}$)	16 ºC	-	Chlorine (0.8 $mg.L^{-1}$)	Tap water microorganisms	Doutorelo et al. (2013)
Packed beads column	Glass, SS and Teflon	Water flow: 1 $L.min^{-1}$	16.0, 19.4 and 20.6 ºC		Chlorine (0.5 $mg.L^{-1}$)	Tap water microorganisms	Delahaye et al. (2006)