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Drinking water biofilm monitoring by Propella™ and Flow Cell bioreactors under different operating conditions

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Abstract

Monitoring of biofilm subjected to different process conditions was performed using two distinct bioreactors, Propella™ and flow cell system. Biofilms were grown on polyvinyl chloride (PVC) and stainless steel (SS) coupons under laminar (Reynolds number of 2000) and turbulent (Reynolds number of 11000) flow. The parameters analyzed were culturable cells, using R2A, and total bacteria, which were assessed using a DNA-binding stain coupled with epifluorescence microscopy. The impact of the different operating conditions in the studied parameters was established after the biofilms reached the steady-state. It was found that the biofilm steady-state was achieved 3 d after the starting of operating conditions for turbulent flow and for both bioreactors and adhesion surfaces. Under laminar flow it was only achieved 6 d after. The number of total bacteria was invariably higher than the culturable cells. The number of total and culturable bacteria in turbulent flow-generated biofilms were similar in both bioreactors, regardless the adhesion surface tested. Under laminar flow, the Propella™ bioreactor allowed the formation of steady-state biofilms with a higher number of total and culturable bacteria than those from the flow cell system. Comparing the effect of the flow regime on biofilm accumulation, only turbulent flow-generated biofilms formed on the flow cell system had a higher amount of total and culturable bacteria than those formed under laminar flow. In terms of adhesion surface effect on steady-state biofilms, a higher number of total and culturable cells were found on PVC surfaces comparatively to SS when biofilms were formed using the flow cell system. Biofilm formation on PVC and SS was similar in the Propella™ system for both flow regimes.

1-Introduction

The dynamics of the microbial growth in drinking water networks is very complex, as a large number of interacting processes are involved. Many problems in drinking water distribution systems (DWDS) are microbial in nature, including biofilm growth, nitrification, microbial mediated corrosion, and the occurrence and persistence of pathogens (Camper 2004; Emtiazi et al. 2004). Biofilms are suspected to be the primary source of microorganisms in DWDS that are fed with treated water and have no pipeline breaches, and are of particular concern in older DWDS (Simões et al. 2006). By adopting this sessile mode of life, biofilm-embedded microorganisms enjoy a number of advantages over their planktonic counterparts. One advantage is the ability of the extracellular polymeric matrix, they excrete, to capture and concentrate a number of environmental nutrients, such as carbon, nitrogen and phosphate (Simões et al. 2006). Another advantage to the biofilm mode of growth is that it enables resistance to a number of control strategies (Simões et al. 2005). DWDS disinfection with chlorine dioxide and chlorine, for example, can reduce the concentration of planktonic bacteria, but have little to no effect on the concentration of biofilm bacteria (Gagnon et al. 2005). This inherent resistance to antimicrobial factors is mediated through very low metabolic levels and drastically downregulated rates of cell division of the deeply embedded

microorganisms. Biofilms act as a diffusion barrier, slowing down the penetration, to some antimicrobial agents (Simões et al. 2005). Other important advantage of the biofilm mode of growth is the potential for dispersion via detachment. Under the direction of fluid flow, detached microorganisms travel to other regions to attach and promote biofilm formation on clean areas (Codony et al. 2005).

Drinking water pipes inner-surfaces are invariably colonized by biofilms, regardless the presence of a residual disinfectant. In addition to the possibility of causing corrosion, taste and odour problems, biofilms control the microbiological contents of the distributed water and are a potential source of pathogens (Szewzyk et al. 2000). In fact, biofilms formed within potable-water systems contain bacterial pathogens such as *Legionella pneumophila* and coliforms of intestinal and nonintestinal origin (World Health Organization 1993). Furthermore, protozoa are commonly found within water distribution systems and have been associated with the persistence and invasiveness of pathogens (Simões et al. 2007a). Such findings implicate the importance of maintaining a continuous residual disinfectant in DWDS. Moreover, the examination of a DWDS reveals the complexity of such a technical system. There are not only many different materials used for the transportation and regulation of the water flow but also dramatic variations in the flow conditions between different locations. Obviously, microorganisms face a diversity of habitats with distinct physicochemical and nutritional conditions during treatment, storage, and distribution of drinking water.

The purpose of the present study was to evaluate the drinking water biofilm formation by drinking water autochthonous bacteria on stainless steel and polyvinyl chloride, two support materials commonly used on drinking water networks, under different water flow rates, using the Propella™ bioreactor and the flow cell system.

2-Material and Methods

2.1-Bioreactors and biofilm monitoring

In this study, monitoring of drinking water biofilm subjected to different conditions was performed using two distinct bioreactors, flow cell system and Propella™. The configurations of these bioreactors are presented in Figure 1.

The drinking water source was from the public network in Braga (North of Portugal). Briefly, tap water was collected in a reservoir, which was connected to one of two consecutive granular activated carbons (GAC) filter columns. It has been shown elsewhere that the first GAC filter eliminates free chlorine and biodegradable matter contained in the tap water, while the second is a biological activated filter providing a continuous bacterial inoculum to the bioreactor (Morin and Camper 1997). To avoid the presence of large carbon particles released from the columns, two filters (pore sizes 20 µm and 5 µm) were placed between the second GAC filter and the mixing tank. This tank supplied a constant inoculum at a flow rate of approximately 0.02 L h^{-1} into each of the flow cells or 1.12 L h^{-1} into the Propella™, in order to obtain the adequate dilution rate and similar to both bioreactor systems. Absence of free chlorine in the mixing tank was certified by regular sampling, using the free chlorine ion specific meter HI-93701 (Hanna Instruments, USA).

Biofilms were grown on polyvinyl chloride (PVC) and stainless steel ASI 316 2R (SS) coupons. The water flow rate through the bioreactors was controlled by recirculating the water by means of recirculation pumps (flow cells) or by means of motor for water agitation (Propella™). The biofilms were developed under laminar (Reynolds number of 2000) and turbulent (Reynolds number of 11000) flow rate. Temperature in the both bioreactors was maintained at 20 ± 1 ºC by an external refrigeration mechanism (Thermomix® BU, B. Braun – Biotech SA).

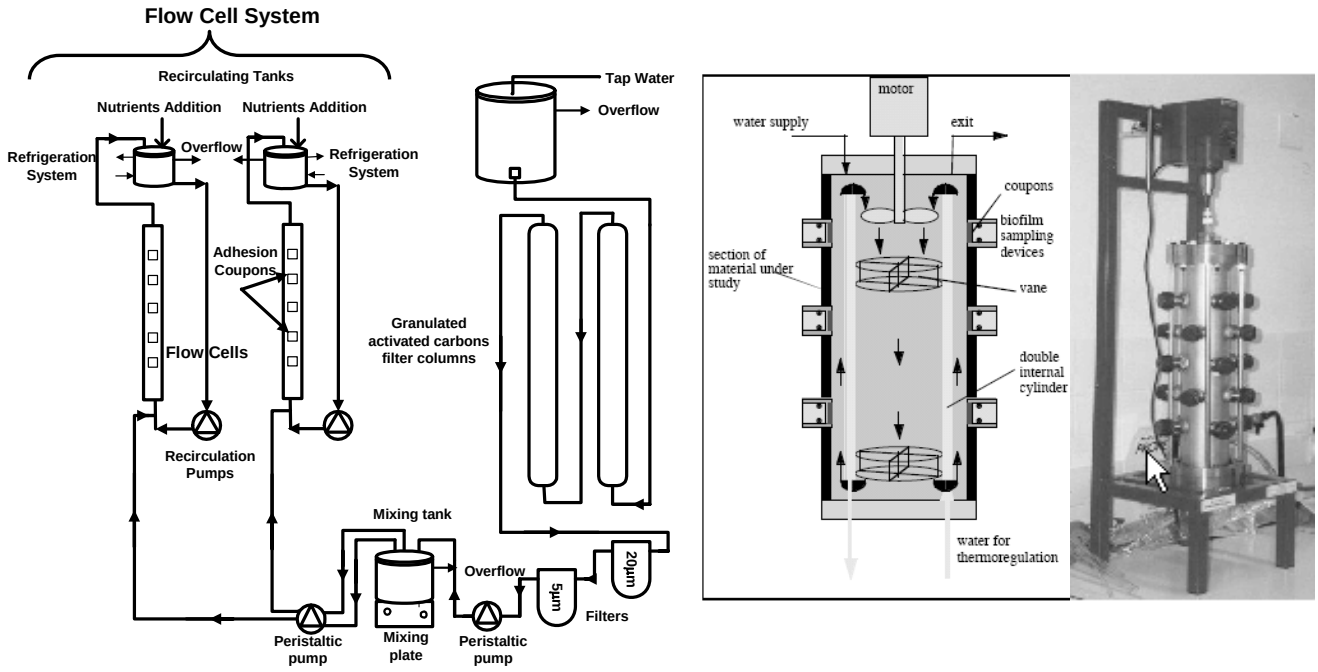

Figure 1 – Experimental set-ups, showing the GAC filter columns and the flow cell system (a); and the Propella^{TM} bioreactor (b).

2.2-Biofilm sampling

Biofilm sampling was made from the top to the bottom of the bioreactors under aseptic conditions and the coupons removed were substituted with new ones that were previously cleaned, immersed in ethanol (70 % v/v) for 30 min, and rinsed in sterile distilled water. The removed coupons were gently washed with sterile saline phosphate buffer (pH=7.0) to remove loosely attached microorganisms and scraped with a scalpel into 15 mL glass tubes containing 10 mL of sterile phosphate buffer. Before serial dilutions, biofilm suspensions in the tubes were vortexed for 2 min and used to assess both colony forming units (CFU) and total cell counts (TB).

2.3-Culturable and total cell counting

CFU`s were evaluated by standard culture methods on R2A (Oxoid, UK) prepared according to manufacturers instructions. Triplicate plates were used for each dilution and for each tested biofilm. CFU's were counted after 15 d of incubation at 20 ± 3 ºC, and the results were expressed as CFU cm^{-2}. TB were obtained by filtering the adequate volume (up to 10 mL as a function of the bacterial concentration) through a 25 mm black Nucleopore® polycarbonate membrane with a pore size of 0.2 µm (Whatman, UK). Before the filtration step, 2 % (v/v) formaldehyde (Merck, Germany) was added to the solution for sample fixation and preservation. After filtration, cells in the membrane were stained with 100 µg mL^{-1} of 4,6-diamino-2-phenylindole (DAPI) (Sigma, Portugal) for 5 min and the preparations were stored at 4 °C for up to 7 d in the dark, before visualization. No significant decay of fluorescence was noticed during this time span. Cells were visualised under an epifluorescence microscope (Carl Zeiss, Germany) equipped with a filter sensitive to DAPI fluorescence (359 nm excitation filter in combination with a 461 nm emission filter). A total of 20 fields were counted and the average of three membranes was used to calculate total cells per cm^2.

2.4.-Statistical analysis

Paired t-test analyses were performed to estimate whether or not there was a significant difference between the results obtained. Statistical calculations were based on confidence level equal or higher than 95%.

3-Results and Discussion

Biofilms constitute a protected mode of growth that allows microorganisms to survival in hostile conditions, being their phenotype significantly different from their planktonic counterparts. Their development, behavior and population characteristics are strongly influenced by many environmental factors and by intrinsic biological properties (Sauer and Camper, 2001; Purevdorj et al. 2002). From the most important environmental factors affecting biofilm structure and behaviour are the velocity field of the fluid in contact with the microbial layer and the support material for bacterial adhesion and further biofilm development (Vieira et al.1993; Stoodley et al.1999; Simões et al. 2006). Hydrodynamic conditions will determine the rate of transport of cells, oxygen and nutrients to the surface, as well as, the magnitude of shear forces acting on a developing biofilm (Vieira et al. 1993). Regarding the effects of the support material, microbial attachment will occur most readily on surfaces that are rougher, more hydrophobic, and coated by surface conditioning films (Donlan, 2002).

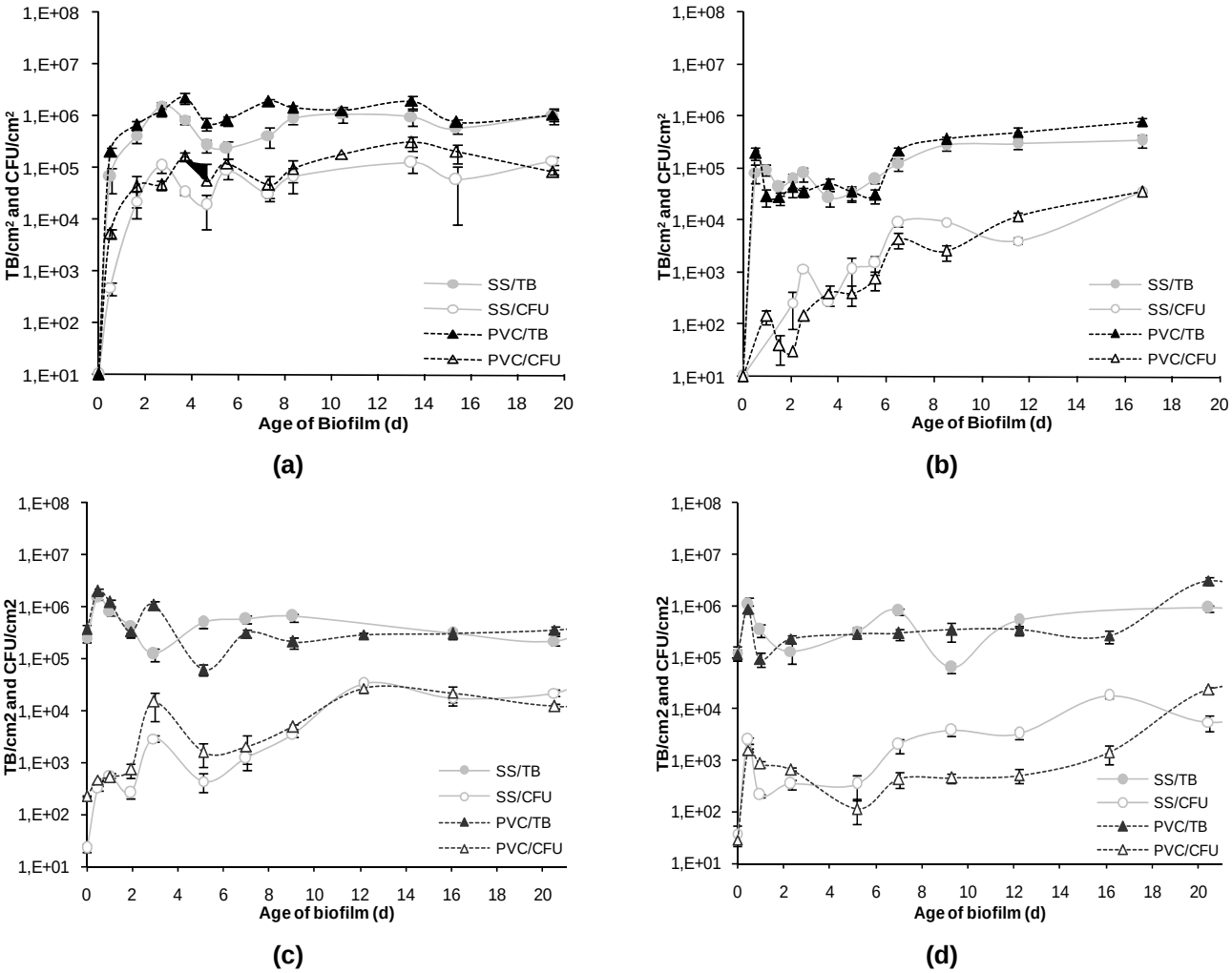

Figure 2 – Kinetics of biofilm growth obtained for the different conditions with the two bioreactors. Biofilm accumulation along time (assessed by TB and CFU) on SS and PVC surfaces. (a) turbulent flow and (b) laminar flow in flow cell bioreactor. (c) turbulent flow and (d) laminar flow in the Propella^{TM} bioreactor.

Biofilm accumulation in all experiments, expressed both in CFU and TB, increased markedly in the first few days, following a sigmoidal curve (Figure 2). Biofilm steady-state was achieved 3 d after the starting of operating conditions for turbulent flow conditions and for both bioreactors and adhesion surfaces. Under laminar flow conditions, it was only achieved 6 d after. For those cases, the number of total bacteria was invariably higher than the culturable cells (differences always higher than 2 log). It has long been recognized that the use of culture-based enumeration techniques may significantly underestimate the numbers of viable cells. Several reasons may account for this difference: the presence of starved or injured cells or potentially viable but nonculturable cells (VBNC) that are not able to initiate cell division at a sufficient rate to form colonies; inadequate culture conditions; aggregation of bacteria that can lead to the formation of one colony from more than one cell, thereby underestimating the total number of cells (Banning et al. 2002). However, total and culturable bacteria in turbulent flow-generated biofilms were similar in both bioreactors, regardless the adhesion surface ($p > 0.05$). This result suggests that increased hydrodynamic stress favours biofilm bacteria culturability. Vieira et al. (1993) found that mass transfer limitations existed in a higher extent in biofilms formed under laminar flow than for turbulent conditions. Consequently, the higher oxygen rate and transport of substrate, even if at very low levels in DWDS, from the fluid to the biofilm (mass transfer effects), should favour microbial metabolism and cell replication. Comparing the effect of the flow regime on biofilm accumulation, it was only found for the flow cell system that turbulent flow-generated biofilms had a higher amount of total and culturable bacteria than those formed under laminar flow ($p < 0.05$). This result is in agreement with previous studies (Stoodley et al. 1999; Simões et al. 2007b), with single and mixed species biofilms formed on flow cell systems, showing that biofilms formed under turbulent flow had a significant higher cell density than the laminar counterparts. Turbulent and laminar flow-generated biofilms formed on the Propella™ bioreactor had comparable cell densities. Moreover, the Propella™ system allowed the formation of steady-state laminar flow-generated biofilms with a higher number of total and culturable bacteria than those formed on the flow cell system ($p < 0.05$). In fact, there are significant differences on the design of the used bioreactor systems that can account for the differences obtained. For example, the hydrodynamic stress is obtained by distinct mechanisms when using Propella™ bioreactor (agitation by means of a rotating device system) and the flow cell system (fluid flow). In terms of adhesion surface effects, in the flow cell system, a higher number of cells formed biofilms on PVC surfaces comparatively to SS ($p < 0.05$), while biofilm formation on PVC and SS was similar ($p > 0.05$) in the Propella™ system, for both flow regimes. In a previous study (Simões et al. 2007c), it was demonstrated that the tested materials had similar physico-chemical characteristics, such as hydrophobicity, and both are prone to colonization by drinking water isolated bacteria. Consequently, it was expected a low biofilm data variability as a consequence of adhesion surface differences.

3-Conclusions

The development and validation of reliable biofilm monitoring techniques is required in order to mimic real environmental situations using laboratorial systems. This work demonstrates that distinct bioreactor configurations provide different biofilm data. In fact, the use of PVC or SS as adhesion surfaces and distinct hydrodynamic conditions lead to biofilm accumulation variability in terms of CFU and TB when using the Propella™ or the flow cell bioreactors. Moreover, this study highlights the need for a deeper understanding of how the large spectrum of conditions interact and affect biofilm formation potential and accumulation with the final purpose of predicting the total and culturable bacteria attached to real drinking water distribution pipes, based on the system characteristics. Although the practical use of these conclusions by drinking water network companies is still limited, the information provided here might be used as a framework for future studies.

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