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The control of biofilm formation by hydrodynamics of purified water in industrial distribution system

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ABSTRACT

Systems for storage and distribution of purified water at ambient temperature are highly susceptible to microbial contamination. The water flow, microbial content and chemical quality of the purified water in an industrial water system have been simulated in a biofilm annular reactor (BAR) to study the impact of different hydrodynamic conditions on biofilm development. Our results reveal the potential of stagnant purified water at total organic compounds (TOC) below 50 ppb to develop biofilm that allows detachment of planktonic bacteria and colonization of new surfaces within 24 h. However, under constant water flow over 7 days, the growth of initial biofilm was 40 times less, fewer bacteria were detached, and new surfaces were colonized to a lesser extent. Heterotrophic plate counts (HPCs) in biofilm were highly positively correlated with numbers of detached planktonic bacteria in effluent water. The study shows that the hydrodynamic conditions and level of planktonic HPC in water are critical for the development of biofilm at very low TOC. The results in the BAR agreed well with those from regular industrial microbial monitoring of purified water. To conclude, the BAR successfully simulates biofilm growth and can be used to establish an effective biofilm control strategy. However, the microbial quality of purified water in industrial system is a constant challenge; any increase of HPC in effluent water is a sign to take steps against excessive microbial growth.

1. Introduction

Purified water is one of the most widely used raw materials in pharmaceutical areas. Deterioration of the microbial quality of water can affect the quality and safety of the products (Hallam et al., 2001; Adley and Saieb, 2005; Simoes et al., 2010). Microorganisms can live and proliferate as individual cells or they can attach to surfaces, where they grow as highly organized multicellular communities or biofilms. Biofilm is the predominant mode of microbial life in nature as well as in persistent chronic infective diseases (Costerton et al., 1999; Davey and OToole, 2000; Estrala et al., 2009; Meng-Ying et al., 2009; Ammons, 2010). Biofilm infections on indwelling devices or implants are difficult to eradicate because of their much better protection against macrophages and antibiotics, compared to free living cells, leading to severe clinical complications often with lethal outcome (Estrala et al., 2009).

The transition of microorganisms from free-swimming cells to a surface-attached community-based lifestyle proceeds via distinct steps, culminating in the formation of a complex structural arrangement of cells: planktonic, attached, structured in microcolony or macrocolony, and detached (Monds and OToole, 2009). Communication between cells via cell–cell signalling is suggested to play a significant role in coordinating cell attachment and detachment (Dunny et al., 2008; Irie and Parsek, 2008).

Biofilm constitutes a protected mode of growth that allows microorganisms to survive, even in hostile environments, their phenotypes, physiology and behaviour being significantly different from their planktonic counterparts (Stewart and Franklin, 2008; Heffernan et al., 2009). Not only do bacteria in biofilm differ from planktonic bacteria of the same species, but also bacteria in mono species biofilm demonstrate vast heterogeneity in terms of metabolism, gene expression and physiology due to the different conditions on different microlocations (Koh et al., 2007; Vlamakis et al., 2008). Much progress has been made in the last decade in elucidating the molecular mechanisms of bacterial adhesion and understanding the structure and composition of biofilm (Karen, 2008; Monds and OToole, 2009).

Biofilm in water systems act as a reservoir of microorganisms – including pathogens, if present in the water (Attman et al., 2009) – that are released sporadically into the water, causing strong increase of cell density. The biological, chemical, and physical factors that drive detachment are complex and incompletely understood (Chambless and Stewart, 2007). Multiple factors are probably associated with attachment and detachment processes,

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depending on the availability of nutrient or oxygen (Chandy and Angles, 2001; Rice et al., 2005), shear-stress (Guillemot et al., 2006; Lee et al., 2008), environmentally controlled exopolysaccharide biosynthesis (Thormann, 2006), an erosive process in which individual cells are lost from the biofilm cell cluster (Bester et al., 2005; Duddu et al., 2009), etc. Recently it has been reported that biofilm structures that reflect changes during its growth, greatly influence the detachment process (Garny et al., 2008; Böl et al., 2008) that is responsible for deterioration of water quality. It is known that cells in biofilm display increased resistance to antimicrobials and environmental stress, causing microbial contamination of water in industrial settings of various processing industries (Hamilton, 2002; Russo et al., 2008; Simoes et al., 2010).

Most studies include disinfection of drinking water, but few are concerned with purified water at ambient temperature (LeChevallier et al., 1996; Sharp et al., 2001; Florjanič and Kristl, 2010; Manuel et al., 2010). Biofilm in systems for storage and distribution of purified water is difficult to detect, inactivate, and remove. In our previous study we reported the impact of two regimens for disinfection of a purified water system with ozone as a function of concentration and time 70 ± 20 ppb in the reservoir in a production regime and 250 ± 20 ppb in the whole system during weekly sanitization. The number of heterotrophic plate counts (HPCs) and the concentration of total organic compounds (TOC) were measured (Florjanič and Krist, 2006). Over four years, 94–98% of water samples exhibited HPC in the category 0–5 CFU/ml, and none in the category $\geq$50 CFU/ml. In spite of increased TOC in the inlet water up to 40 ppb, the microbial counts in purified water in the distribution loop were unaffected. It was emphasized that the critical points regarding microbial contamination of the purified water system are user point valves and the tubes used for transferring water to equipment. The specified ozone level prevented microbial growth and formation of biofilm in the distribution system to an extent that could endanger the water quality and even cause sporadic release of microbes to the water.

Our understanding of biofilm physiology and micro-ecology originates from experiments using *in vitro* biofilm models. Broadly speaking, such models may be used to replicate conditions within the laboratory or to focus on selected variables such as the impact of fluid hydrodynamics, nutrient concentration and antimicrobials on biofilm growth. Today biofilm models, including microtitre plate systems, flow cells, different biofilm fermentors, annular reactor, miniaturized calorimetry, are commonly used (Böl et al., 2008; Duddu et al., 2009; Lee et al., 2008; Karen, 2008; Lerchner et al., 2008; Russo et al., 2008).

Although the use of antimicrobial agents is widespread in biofilm control (Simoes et al., 2010), there are no standardized quantitative methods for selecting antimicrobials or for the design of efficient biofilm control protocols of pharmaceutical waters. Even in current United States and European pharmacopoeias, no biofilm sampling is defined or required for microbial quality determination of purified water. Consequently, the approach to design the system and operating characteristics should be developed for each particular case separately (Russo et al., 2008). With this in mind, we reviewed the experimental approaches used for drinking water (LeChevallier et al., 1996; Sharp et al., 2001; Ndiongue et al., 2005). A relatively new laboratory method for investigating biofilm in drinking water involves the biofilm annular reactor (BAR). No study of biostability of purified water using a BAR has been published.

In this study the impact of water hydrodynamics and residence time on biofilm growth, detachment of bacteria, colonization of new surfaces and dependence on numbers of planktonic and biofilm bacteria initially present in water, have been investigated. The industrial purified water hydrodynamics were simulated in a BAR as closely as possible. Additionally, the results obtained with the BAR were compared with the past 2 year's HPC results of regular microbial monitoring of purified water in an industrial system. The results focus on the impact of water hydrodynamics on biofilm growth and behaviour, and on possible quality assurance strategies.

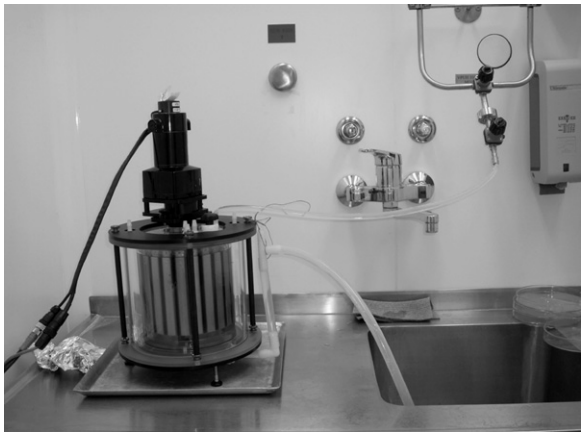

Fig. 1. Biofilm annular reactor (BAR) connected to the industrial purified water system.

2. Materials and methods

2.1. Design and operation of the biofilm annular reactor (BAR)

The BAR, model 1320 (BioSurface Technologies, Bozeman, Montana), consists of two cylinders: a stationary outer made of glass and a metal inner cylinder rotating on its vertical axis at up to 200 rpm (Fig. 1). The inner cylinder is equipped with 20 removable coupons with surface area 15 cm^2, of the same quality as the material of the water system. The BAR has a working volume of approximately 1 L and a high surface area to fluid volume ratio. Before experiments the tubs and the BAR were thoroughly cleaned by laboratory detergent, assembled and sterilized by moist heat at 121 °C for 1 h. The stainless steel coupons were sterilized by dry heat at 300 °C for 2 h and installed in the reactor aseptically. The BAR was connected by a tube directly to the industrial purified water distribution system. Sanitization of an industrial purified water storage and distribution system was performed with ozone in 7 days periods (Florjanič and Krist, 2006).

Purified water enters through the inlet opening on the top and, after moving in the region between both cylinders, leaves the equipment through the outlet at the bottom. While passing between cylinders the cells can attach and form biofilm on the sterilized coupons. In operation, the flow rate of the inlet water was 10 or 120 ml/min, which enable residence times less than 2 h (dilution rate of <0.5/h). During experiments samples of influent and effluent water and as well as the biofilm on coupons were monitored at known TOC, conductivity, temperature and pH of water.

2.2. Impact of hydrodynamic conditions on biofilm growth in BAR

The impact of four different incubation times and two different operation regimes on biofilm growth in BAR was investigated. Each experiment consists of a period in which water is stationary in the BAR (incubation time) and a period in which the water flows through the BAR and the inner cylinder is rotating (operating regimes) (Table 1). Incubation times in EXP1, EXP2, and EXP3a were 96, 72 and 24 h followed by operation regime 1 for 7 or 14 days. In EXP 4, water flowed constantly through the BAR in operation regime 2 without prior incubation. The indicators of biofilm growth were: density of HPC on the surface of coupon (CFU/coupon)