*Chapter*

# BIOFILMS IN DRINKING WATER

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## ABSTRACT

The provision of safe drinking water (DW) is a top priority issue in any civilized society. Safe DW is a basic need to human development, health and well-being. The main challenge to the DW industry is to deliver a product that is microbiologically and chemically safe, aesthetically pleasing and adequate in quantity and delivery pressure. Normally, the water that leaves a treatment station has quality, but its quality decreases along the travel in the drinking water distribution systems (DWDS). Water industries and governments over the world are working together in order to improve DW quality through the effective treatment, monitoring of its physicochemical and microbiological properties, and the design and the operational management of the distribution networks. Although DW is strictly monitored in developed countries, waterborne outbreaks are still being reported due to microbial contamination. Biofilms contribute notoriously to these events, creating a protective and nutritional reservoir for pathogens growth and survival. Nevertheless, the dynamics of microbial growth in DW networks is very complex, as a large number of interacting processes (physicochemical and biological) are involved. DW biofilms constitute one of the major microbial problems in DWDS that most contributes to the deterioration of water quality. Although biofilm elimination from DWDS is almost impossible, several aspects can be manipulated in order to prevent and control their growth. This book chapter provides a contribution to better understand the important biological and ecological mechanisms involved in biofilm formation in DWDS, with intent to control and prevent their formation, in order to improve DW quality that reaches to consumer's tap.

**Keywords**: Biofilm; Drinking water; Public health

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## INTRODUCTION

The main goal of water companies is to deliver to each consumer microbiological safe drinking water (DW), adequate in quantity and delivery pressure and acceptable in terms of taste, odour and appearance. Studies in a full-scale drinking water distribution system (DWDS) indicated that most bacteria derived from the biofilm of pipeline surfaces. DWDS are known to harbour biofilms, even in the presence of a disinfectant. Biofilms are constituted by a microbial community adapted to conditions of low nutrient concentration and high disinfectant levels. The presence of biofilms in DWDS constitutes one of the currently recognized hazards affecting the microbiological quality of DW and may lead to a number of unwanted effects on the quality of the distributed water [1]. Microbial growth may affect the turbidity, taste, odour and colour of the water, contribute to the increase of the amount of cells in the bulk phase, promote the deterioration of metallic pipes, induce a disinfectant demand and therefore promote disinfectant decay in the distribution system [2]. Also, biofilms can constitute a reservoir of pathogenic microorganisms, which are responsible for several waterborne diseases [1, 3-4].

The development of biofilms in DWDS is influenced by several factors, including microbial quality of intake water, concentration of biodegradable organic matter, amount of available nutrients, sediment accumulation, concentration of residual disinfectants, water residence time, environmental factors (pH, temperature and turbidity of the water), design of network (presence of dead ends, diameter of pipes), hydrodynamics (shear stress at the biofilm-liquid interface), characteristics of material covering the distribution pipes (composition, porosity, roughness) and their conservation state [2]. Recent studies into the microbial ecology and population dynamics of DWDS have found that other important mechanisms play a determinant role in DWDS biofilm formation and on their resistance to disinfectants. Those include the microbial diversity, interspecies interactions, autoaggregation and coaggregation, presence/release of microbial metabolites and molecules (cell-cell signalling), and transfer of genetic material [2]. However, the role of those mechanisms in DWDS biofilm formation remains poorly understood. The purpose of this book chapter is to provide new and relevant information on the role and mechanisms (physicochemical and biological) of biofilm formation in DWDS.

## BIOFILMS IN DRINKING WATER DISTRIBUTION SYSTEMS

### Biofilms: Definition and their Impact

In general, a biofilm can be defined as a community of microorganisms that is irreversibly attached to a biotic or abiotic surface and that is enclosed in a matrix of exopolymeric products [5-6]. DW biofilms, particularly, are composed by complex microbial communities functionally organized and embedded in a gelatinous matrix of extracellular polymers excreted by microorganisms (Figure 1a). Extracellular polymers also known as extracellular polymeric substances (EPS) are the key substances keeping biofilm organisms together, gluing them to the surface and providing protection against agents of stress. Any inorganic particle passing nearby (e.g. corrosion products, clays, sand, etc.) may also be

incorporated in the biofilms (Figure 1b) increasing its “mechanical strength” [7-8]. According to Characklis and Marshall [9], bacteria are generally dominant in whatever biofilm due to their high growth rates, small size, adaptation capacities and the ability to produce EPS. However, virus, protozoa, fungi and algae may also be present in DW biofilms as reported by several authors [10-14].


Figure 1. (a) Scanning electron microscopy (SEM) photomicrographs of 24 hours old biofilms formed by the opportunistic Gram-negative *Burkholderia cepacia* (isolated from laboratorial DWDS) evidencing the presence of an extracellular polymeric matrix (× 15000 magnification; bar = 2 µm). (b) Ductile iron pipe section from a DWDS with biofilm and high amounts of corrosion products. This section of DWDS was obtained as result of a pipe break in the DWDS.

Biofilms are well organized structures where microorganisms are protected from environmental stress and allow complex interactions among different species, i.e. antagonistic or synergistic relationships [15-18]. In biofilms, the way that cells communicate and organize in a social community is controlled by the secretion of signalling molecules in a process called “quorum sensing”. This promotes communication between cells and regulates the relationship between cells resulting in a group behaviour instead of an individual performance [19-21].

## Relevance of Biofilms in Water Industry

The biofilms in DWDS, also designated as biofouling, are a well-recognized problem in water industry. Biofouling, in general, refers to the undesirable accumulation of biotic matter on a surface. It has been shown to be of considerable hygienic, operational and economical relevance, not only in DWDS but also in other purified water supply systems [1].

Many problems in DWDS are microbial in nature, including biofilm growth, nitrification, microbially-mediated corrosion and the occurrence and persistence of pathogens [22-26]. 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 [27-28]. Flemming *et al.* [29] estimated that 95% of the overall biomass is attached to pipe walls, while only 5% is in the water phase. Therefore, the microbial growth in biofilms is highly relevant for water quality since they may directly affect cell density in the bulk phase.

By adopting this sessile mode of life, biofilm-embedded microorganisms enjoy a number of advantages over their planktonic counterparts, namely: the ability to excrete the EPS matrix to capture and concentrate nutrients; resistance to a number of removal strategies such as antimicrobial and antifouling agents as well as shear stress conditions; the possibility of metabolic interactions between bacteria with different physiological requirements; bacterial communication through excreted signalling molecules and the potential for dispersion via detachment, maintaining a persistent bacterial source population that is resistant to antimicrobial agents, while at the same time enabling continuous shedding to promote bacterial spread [1].

The current knowledge of the structure and activities in biofilm communities is still limited, because analysis of microbial physiology and genetics have been largely confined to studies of microorganisms from few lineages for which cultivation conditions have been determined and for some process conditions, not mimicking real environments. The dynamics of the microbial growth in DW networks is very complex, as a large number of interacting processes are involved. DW pipes inner-surfaces are invariably colonized by biofilms, regardless of 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 [30-31]. The interaction of pathogens with other biofilm microorganisms has been a principle of concern in man-made water systems, particularly DWDS. In fact, biofilms formed within potable-water systems contain bacterial pathogens such as *L. pneumophila* and coliforms of intestinal and non-intestinal origin [28,32-33]. Furthermore, protozoa are commonly found within DWDS biofilms and have been associated with pathogen persistence and invasiveness [34-35]. Despite Payment *et al.* [36] in their work did not find any relationship between biofilm presence in DWDS and occurrence of disease, it has been proved that pathogens such as *L. pneumophila*, *Mycobacterium* spp., *P. aeruginosa*, *Klebsiella* spp., *Burkholderia* spp., *Giardia* and *Cryptosporidium*, among others (Table 1), are transmitted by contaminated water and biofilms are a good candidate as they can act as a protective niche for their survival in DW as shown by several authors [31, 37-38]. The consumption of contaminated DW can cause a wide range of diseases and health-related problems in all people or in more susceptible groups like infants, young children, elderly or sick or immune-compromised people. Waterborne diseases are any illness caused by the utilization of DW contaminated by human or animal faeces, which contain pathogenic microorganisms, or by chemical products. Waterborne pathogens are disease-causing bacteria, protozoa, virus and helminths that are transmitted to people when they consume untreated or inadequately treated water. A list of the most relevant agents can be found in Table 1. If these pathogenic microorganisms are not removed by disinfection and reach the consumer's tap, they may cause outbreaks of disease within the community. The occurrence of outbreaks of waterborne diseases is not limited to developing countries; affluent countries are also affected [39-42]. Such findings demonstrate the essential role of an efficient disinfection plan to control microorganisms in the bulk phase and their biofilms in order to provide high quality DW [1].

**Table 1. Pathogens associated to waterborne diseases**

| **Bacteria** | **Protozoa** | **Viruses** | **Helminths** |
|---|---|---|---|
| *Acinetobacter* spp. | *Acanthamoeba castellani* | Adenovirus | *Ascaris lumbricoides* |
| *Aeromonas* spp. | *Balantidium coli* | Astrovirus | *Dracunculus medinensis* |
| *Burkholderia pseudomallei* | *Blastocystis hominis* | Coxsackie vírus A | *Fasciola* spp. |
| *Campylobacter coli* | *Cryptosporidium parvum* | Coxsackie vírus B | *Schistosoma* spp. |
| *Escherichia coli* pathogenic | *Cyclospora cayetanensis* | Echovirus | Free-living nematodes other than *Dracunculus medinensis* |
| *E. coli* enterohaemorrhagic | *Entamoeba histolytica* | Enterovirus | |
| *Francisella tularensis* | *Giardia duodenalis* | Hepatite A virus | |
| *Helicobacter pylori* | *Giardia intestinalis* | Hepatite E virus | |
| *Klebsiella* spp. | *Giardia lamblia* | Norovirus | |
| *Legionella pneumophila* | Microsporidia | Poliovirus | |
| *Leptospira* spp. | *Naegleria fowleri* | Rotavirus | |
| *Mycobacterium* spp. (non-tubercolous) | *Sarcocytis* spp. | Sapovirus | |
| *Pseudomonas aeruginosa* | *Toxoplasma gondii* | | |
| *Salmonella typhi* | | | |
| *Salmonella paratyphi* | | | |
| *Salmonella* spp. | | | |
| *Shigella* spp. | | | |
| *Staphylococcus aureus* | | | |

**Table 1. Pathogens associated to waterborne diseases**

| Bacteria | Protozoa | Viruses | Helminths |
|---|---|---|---|
| Toxic *cyanobacteria* | | | |
| *Tsukamurella* spp. | | | |
| *Vibrio cholera* | | | |
| *Yersinia enterocolitica* | | | |

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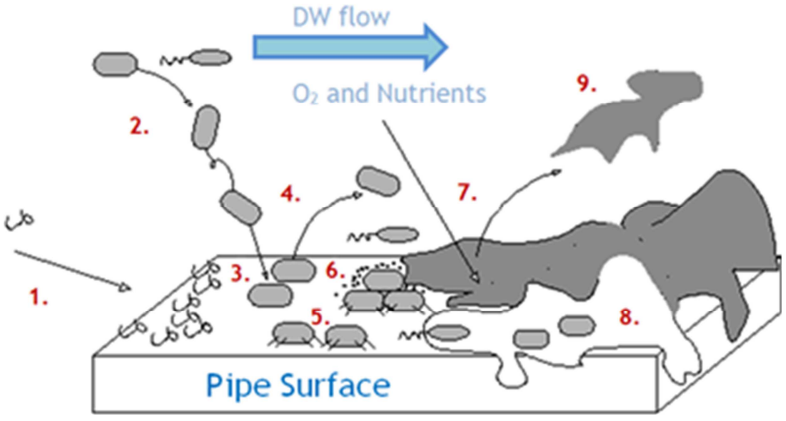
## Biofilm Formation: Physical, Chemical and Biological Processes

The biofilm formation mechanisms were already described extensively, there are several excellent comprehensive reviews on this topic [43-48]. There are a number of mechanisms by which numbers of microbial species are able to come into closer contact with a surface, attach firmly to it, promote cell-cell interactions and grow as a complex structure [47]. Biofilm formation is a dynamic process and comprises a sequence of steps. Currently, processes governing biofilm formation that have been identified include the following steps (Figure 2) [47, 49]:

1) preconditioning of the adhesion surface either by macromolecules present in the bulk liquid or intentionally coated on the surface;
2) Transport of planktonic cells from the bulk liquid to the surface;
3) Adsorption of cells at the surface;
4) Desorption of reversibly adsorbed cells;
5) Irreversible adsorption of bacterial cells at a surface;
6) Production of cell-cell signalling molecules;
7) Transport of substrates to and within the biofilm;
8) Substrate metabolism by the biofilm-bound cells and transport of products out of the biofilm. These processes are accompanied by cell growth, replication, and production of EPS;
9) Biofilm removal by detachment or sloughing.




Adapted from Simões *et al.* [49].

Figure 2. Processes governing DW biofilm formation: (1) Preconditioning the pipe surface by macromolecules (organic and inorganic) present in the water; (2) Transport of planktonic cells from water to pipe surface; (3) Adsorption of cells at the pipe surface; (4) Desorption of reversibly adsorbed cells; (5) Irreversible adsorption of cells; (6) Production of QS molecules; (7) Transport of substrates to and within the biofilm; (8) Substrate metabolism by the biofilm-bound cells and transport of products out of the biofilm, accompanied by cell growth, replication, and production of EPS; (9) Biofilm removal by detachment or sloughing.

***Conditioning Film***

The first step in biofilm formation (step 1) is the preconditioning of the adhesion surface. The conditioning film is a thin layer of organic molecules and ions covering the adhesion surface that is formed before any microorganisms attach to the surface. These molecules may adhere to the surfaces by physical or chemical adsorption. Physical adsorption is generally a reversible process in which one monolayer is formed, involving nonspecific bonds (London and van der Waals forces). In chemical adsorption, several adsorbed molecular layers are formed involving specific chemical bonds (electrostatic, covalent and hydrogen bonds), dipole interactions, and hydrophobic interactions [50]. The strength of biofilm adhesion is largely dependent on the cohesion of the conditioning as observed by several authors [51-52].

***Adhesion***

Steps between 2 and 5 correspond to the effective adhesion of microorganisms to surfaces. This is started by the transport of microbial cells to the adhesion surface either by fluid dynamics, gravitational forces and Brownian motion, or by migration through active cell motility (e.g. flagella). Also, the surface electrostatic charge and hydrophobic interactions affect this approaching and the adhesion process. When the cells approach the surface they can interact with each other by the establishment of long and short/intermediate distance forces. The long distance forces are described by the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory and comprise the attractive forces of van der Waals and the repulsive forces of the electrostatic double-layer. The short/intermediate distance forces include hydrophobic interactions, hydrophobic pressure, steric forces, Born repulsion forces and polymer bridges [53-54]. In equilibrium, when favourable, this results in the adhesion of microorganisms.

***Biofilm Growth and Maturation***

After cellular adhesion to surfaces, the growth and maturation are the following stages of biofilm formation (steps 6-9). The attached microorganisms start growing, they form microcolonies, excreting organic polymers and initiating the formation of the biofilm matrix. Exopolysaccharide synthesis has been shown to be important for the formation of microcolonies [55-56]. As biofilm thickness increases, transport of nutrients from the external liquid media to the inner layers of biofilm and transport of excreted metabolites in the opposite direction are important for biofilm maintenance. Throughout the phase of biofilm growth, bacteria detachment events occur although to a lower extent compared to the growth rate. In the maturation phase, there is the development of a complex and organized consortia of microorganisms embedded in an organic matrix that protects the microorganisms inside from stress factors. It is in this stage that microorganisms produce large amounts of EPS. The structure of a mature biofilm depends on the microbial composition, EPS production, the nutrient availability, hydrodynamic conditions and temperature. In a mature biofilm several processes may occur simultaneously: bacteria detachment into water, attachment of planktonic bacteria, growth and death. However, in this stage these processes are at equilibrium and the number of attached cells per unit surface area is constant in time, although with periodic fluctuations [57-58]. At this phase, the biofilm should reach the highest thickness that is essentially dependent on the hydrodynamic conditions, mass transport and biofilm cohesion.

***Detachment***

The last phase of biofilm formation (step 9) is the detachment of cells and other components from the biofilm. Hydraulic shear stress provoked by high flow velocities can lead to detachment of bacteria and biofilm aggregates (sloughing), with higher detachment rates at increasing shear [59]. Detachment occurs due to different mechanisms: erosion (the continuous release of single cells or small clusters of cells), sloughing (the rapid detachment of large portions of the biofilm), abrasion (collision of solid particles with the biofilm), and predator grazing. Erosion and sloughing can result from biofilm-associated processes, such as enzyme production [60-61], the excretion of certain signalling molecules [62], cell-cycle-mediated events [63-64], and the excretion of surface modified products (surfactants) by certain bacteria [65], or from external factors such as shear forces [64,66], variations in the nutrient concentration [67], chemical change in EPS due to the presence of chelating agents ($Ca^{2+}$) that will reduce the cohesive strength of the attached cells [68], abrasion, and predator grazing [69].

## Biofilm Structure and Composition

The knowledge of biofilm structure allows a better understanding of how developing biofilms are influenced by the surrounding environment and enables better interpretation of biofilm processes. Over time there has been a shift on perception of the structure of microbial biofilms from that of a homogenous layer of cells in a slime matrix to a much more heterogeneous arrangement. So, several structures have been proposed as biofilm visualizing techniques were improved through the years. The first simplifying assumption that probably extended through the 1980's well into the following decade was that a biofilm could be represented as a simple planar structure, largely 2D, with a relatively constant thickness [70]. In the meantime episcopic differential interference contrast microscope was developed by Keevil and Walker [71] and the heterogeneous mosaic model was proposed for biofilms growing on the inner surfaces of DWDS. These researchers discerned stacks consisting of microcolonies of bacteria held together by EPS and appearing as columns surrounded by a liquid phase in which grazing protozoa could be discerned. Below the stacks there was a layer of cells about 5 µm thick attached to the substratum. These types of structures led Bill Keevil to name this the “heterogeneous mosaic model”. Another biofilm structure was proposed by Costerton and co-workers [72-75]. When working with river biofilms supplemented with nutrients, these researchers observed a heterogeneous structure composed of mushrooms with the stalk narrower than the upper surface parts, the whole being penetrated by channels allowing the transportation of water, nutrients and metabolites.

According to Wimpenny and Colasanti [76], who proposed a unifying hypothesis for the microbial biofilm structure based on simple and automaton model, all these conceptual structure models were correct since the final structure was largely dependent on the resource concentration. Thus, the first type was dense relatively uniform biofilm found in habitats where the nutrient levels are generally high (e.g. the human mouth), or periodically extremely high. The second type appeared in water distribution systems where the substrate concentration is very low. The third type was generated in the laboratory using media containing significant nutrient concentration [77]. However, there are reports that indicate the

presence of channels in dental plaque biofilms [78] and describe a dense flat biofilm formed under conditions of phosphate starvation [79].

The biofilm structure can be determined by a great variety of environmental parameters (hydrodynamics, nutrient composition, temperature and pH) that, consequently, affect the density, porosity and thickness [76,80-81]. Most environmental biofilms are heterogeneous microbial communities that have different behaviours depending on the conditions (e.g. exopolymers) and the interactions with each other (e.g. chemotaxis, metabolic interactions), hence forming unique biofilms where all resources and energies are optimized.

The structure of DW biofilms on the pipe surface does not follow a standard rule: they may cover the entire inner surface [82-84] or be formed by dispersal aggregates [71]. The surface coverage degree depend of many factors, such as the type of microorganisms, biofilm age, hydrodynamic conditions, presence of inorganic particles, nutrients and temperature.

As result of the application of advanced microscopy, such as confocal laser scanning microscopy and episcopic differential interference contrast microscopy, molecular and electrochemical high-resolution methods have provided insights into the structural organization and function of biofilm communities. Therefore, a mature biofilm is seen as very heterogeneous arrangement, consisting of microcolonies of bacterial cells encased in EPS matrix separated by water channels [44,85]. But although some structural attributes can generally be considered universal, every microbial community is unique [86]. This is due to the fact that a biofilm structure can be influenced by several conditions, such as surface and interface properties, nutrient availability, the microbial community composition, and hydrodynamics, making the exact structure of any biofilm probably a sole feature of the environment in which it develops [87-89]. The water channels that separated the matrix enclosed microcolonies are vital for biofilm maintenance, providing a nutrient flow system inside it [44], that delivers nutrients deep within the complex community [90] and allows the exchange of metabolic products with the bulk fluid layer [91].

Concerning the biofilm composition, water is considered to be the major component of the biofilm, representing from 70 to 99% while bacteria occupy only between 10 and 50% of the total volume of biofilm [92-94]. EPS, the major component of biofilm matrix, are considered the organic substances excreted by attached microorganisms, account for 50 to 90% of the total organic carbon of biofilms [95] and are important keys for the biofilm start-up [94,96]. Their composition and amount are also highly influenced by the type of microorganisms and environmental conditions such as nutrients, temperature, pH and hydrodynamics. For example, the excess of available carbon and the limitation of other nutrients (nitrogen and phosphate), promote exopolysacharides synthesis [80,96]. The EPS determine the structural and functional integrity of microbial biofilms, and contribute significantly to the organization of the biofilm community [97]. EPS are involved in the formation and maintenance of a 3-dimensional, gel-like, highly hydrated and locally charged biofilm matrix, in which the microorganisms are more or less immobilized.

Besides polysaccharides, proteins, nucleic acids or phospholipids, non-cellular materials such as mineral crystals, corrosion products or blood components, may also be found in the biofilm matrix [82]. The biofilm matrix (composed by all inorganic and organic substances surrounding the cells) has several functions. Furthermore, acting as the structural backbone, biofilm matrix protects bacteria from being washed out, from mechanical shocks, from toxic/lethal attacks by antibiotics [98], disinfection chemicals [92,99], UV radiation [100], predators [71] and from desiccation [80,99,101]. As well promotes the storage of nutrients for

intake during periods of limitation [102], the retention of extracellular enzymes [103], the horizontal gene transfer [104], and the exchange of signalling molecules and metabolites [62].

## Factors Affecting Biofilm Growth in Drinking Water Distribution Systems

The attachment of microorganisms to surfaces and the subsequent biofilm development are very complex processes, affected by several factors, as previously stated. In DWDS these include the nature and concentration of nutrients, sediment accumulation, the type and diversity of microorganisms present and their microbial interactions, concentration of free residual disinfectants, environmental factors (including pH and temperature), water residence time, hydrodynamics conditions, design of network (presence of dead ends, diameter of pipes), characteristics of the material covering the distribution pipes and their age. However, in real systems all these factors work together to influence biofilm accumulation. Thus, the impact of some of them may be insignificant compared with the impact of others and must therefore be considered carefully for each system. During the last decades an extensive research has been done in this topic which resulted into several published reports on the effects of diverse factors in DW biofilm formation [105-111]. The main factors will be briefly described.

### *Environmental Factors (pH and Temperature)*

pH and temperature are considered two important factors affecting life by modifying the electrostatic interactions between surfaces and microorganisms, microbial metabolism, enzymatic activity, kinetics and equilibrium of reactions, and other properties (e.g. diffusivity, solubility) [2]. Also, pH and temperature affect the effectiveness of disinfection. Chlorine residuals present in DWDS are drastically reduced when temperature increases and pH decreases [2].

### *Disinfectant*

Other important variable in biofilm formation is the concentration of disinfectant residual in DWDS. The most used disinfectants are chlorine, chloramines, chlorine dioxide, ozone and UV radiation [112]. From all the disinfectants chlorine is by far the most widely used in DWDS. Chemical disinfection and maintenance of chlorine residual through the distribution systems are almost worldwide strategy to prevent bacterial regrowth during water transportation [113-115]. Even so, regrowth may occur when the chlorine residual decays further down in the distribution system [116-117]. Some studies have demonstrated that chlorine is able to control biofilm formation by reducing the rate of biofilm growth, promoting the biofilm detachment and decreasing the activity of microorganisms [105,115,118-119]. However, the presence of residual chlorine is also one of the stress factors that leads to biofilm formation [120]. Nevertheless, some European countries notably the Netherlands, Germany, Austria and Switzerland have taken the approach of distributing high quality DW without the use of residual chlorine. The control of microbial growth in these countries is obtained through limitation of the nutrients essential for growth by more appropriate DW treatments (sedimentation, filtration, UV disinfection, ozone, peroxide), i.e. by the production of biologically stable DW [1].

***Hydrodynamics***

The hydrodynamic conditions in DWDS are variable. These conditions alternate from laminar to turbulent flow, but stagnant waters also occur in places where the water consumption is low, as well as in reservoirs and buildings. The flow velocity may cause different effects on biofilm accumulation and detachment [96]. Nutrient transport rates within the biofilm increase with the flow velocity until a maximum value is reached, and then decrease as the velocity is further increased. This transport rate promotes bacterial growth within the biofilm. On the other hand, the biofilm density and detachment increase with the flow velocity [96]. As result of wide research on effects of flow velocity on biofilm accumulation controversial results were obtained. Several authors observed that biofilm formation increases with flow velocity [111,121-123], while others achieved the opposite effect [124-126]. A mechanistic explanation about the effects of hydrodynamics on biofilm growth was given by several studies on the biofilm metabolism [127-128]. Higher flow velocities increase the cellular hydrophobicity and will promote cell aggregation and hence biofilm accumulation.

***Nutrients***

Generally, DWDS are considered oligotrophic environments (with low contents of nutrients like carbon, nitrogen and phosphorous). However, the increase of nutrients in water promotes biofilm formation. Studies have shown a positive relationship between the concentration of nutrients in DW and bacterial regrowth in DWDS [129-130]. Several studies from the DWDS around the world observed that the organic carbon content was the limiting nutrient. An increase in this nutrient promoted bacteria regrowth: in Australia [116], France [131-133], USA [27,134], Singapore [135], Spain [136], Netherlands [129] and in China [137]. Batté *et al.* [132] observed that the addition of phosphorous did not affect the accumulation of biofilm although phosphorous was being incorporated in biofilm. Other researchers observed that the limiting nutrient was phosphorous in DWDS from Japan [138] and Finland [139]. The detection of the limiting nutrient in DWDS is very important since the addition of phosphate based compounds has been proposed to prevent pipe corrosion and the bacterial regrowth [31,140-141]. The concentration of nutrients such as carbon/nitrogen ratio is important to the production of extracellular polymers and thus affects the adhesion of microorganisms to surfaces [142].

Hydrodynamic conditions and nutrients are the two main parameters that influence biofilm growth in particular the structure, density and thickness [76,81,143]. High shear stress and nutrient limitations led to thin and dense biofilms that will have reduced internal nutrient diffusion [144-145] and increased resistance to removal and cohesion [57,123,125,146]. Under low flow velocities and high nutrients content, the biofilms grow quickly with a low dense structure but with many pores, channels and protuberances [147].

***Materials***

The variability of materials in DWDS is high. Formerly, the majority of pipelines in DW networks were made of iron-based or cement-based materials. More recently, polymeric materials have been preferred, mainly polyvinyl chloride (PVC), polyethylene (PE), because they are easier to handle and implement. In fact, it is possible to find all this types of materials in the same DWDS. The influence of support materials on biofilm growth is well documented

in the literature [10,148-152]. However, there is still controversy about the effects of surface materials on biofilm development when polymeric and metallic materials were compared. Some reports demonstrated that DW biofilms grew less on polymeric materials than on iron matrices [149-150,153-155]. This fact was attributed to iron corrosion products that favour biofilm protection from mechanical and chemical stresses. Other studies reported higher biofilm formation on PVC and PE surfaces than on galvanized steel materials [148,151,156]. While, other works concluded that there was no significant difference in the colonization of the investigated materials (stainless steel, PVC and PE) after decades of operation [157-159]. Lehtola *et al*. [160] found that biofilms grew faster on PE than on copper pipes, but such differences could not be detected in older piping systems. The main characteristics of materials that have been identified as important on biofilm formation are the roughness and the surface physicochemical properties (chemical composition, solid surface tension, hydrophobicity and surface charge). Another aspect is the leaching of volatile components from pipe materials that can be metabolized by biofilm microorganisms. van der Kooij *et al*. [161] also observed that the polymeric materials in contact with DW could release biodegradable compounds, thus enhancing biofilm formation. Moreover, corrosion resistance of the materials may be another important factor when choosing the material for the DWDS. Corroded iron pipes may offer numerous bacterial attachment sites and bacteria protection from the effect of flow rate and of disinfectants as well as may release undesirable products to the water [150,162]. Also, the corrosion products may retain nutrients (such as, humic matter) for subsequent utilization by biofilm bacteria [163]. The corrosion on metallic surfaces may be induced by the activity of physiologically diverse microbial species within the biofilms [164].

***Microorganisms***

The physiological state and the type of microorganisms present in the bulk water will affect the attachment process, since each microorganism has different surface properties, extracellular appendages and abilities to produce EPS. Cell surface hydrophobicity and the presence of extracellular filamentous appendages may influence the rate and the extent of microbial attachment. The hydrophobicity of the cell surface is important in adhesion because hydrophobic interactions tend to increase with the increase in the non-polar nature of one or both surfaces involved, *i.e.*, the microbial cell and the adhesion surface [82]. According to Drenkard and Ausubel [165], the ability of bacteria to attach to each other and to surfaces depends in part on the interaction of hydrophobic domains.

Many microorganisms produce extracellular filamentous appendages. These may, therefore, play a role in the attachment process. In fact, their radius of interaction with the surface is far lower than that of the cell itself. A number of such structures are known to exist - *flagella*, *pili* or *fimbrae*, *prothecae*, *stalks* and *holdfast* [166]. These structures are responsible for motility, involved in the cell-surface interactions and adhesiveness [8]. EPS produced by microorganisms are responsible for binding cells and other particulate materials together (cohesion) and to the surface (adhesion) [80,167-168]. The general composition of bacterial EPS comprises polysaccharides, proteins, nucleic acids, lipids, phospholipids, and humic substances [80,169-170]. According to Tsuneda *et al.* [171], proteins and polysaccharides account for 75-89% of the biofilm EPS composition, indicating that they are the major components. The type of microorganisms and the interactions established between them has an important role in the biofilm dynamics. The protozoa in DWDS are considered

the major organisms responsible for bacterial grazing, which has been shown to limit biofilm accumulation [13,121,172-175]. However, in contrast to predation, association of several pathogenic bacteria to protozoa is a well recognized phenomenon that promotes high resistance against disinfectants and increases the health risk events [13,157,172-173,175-176].

***Sediment Accumulation***

Sediment can consist of either organic matter, including micro-organisms, or insoluble material, mainly iron and manganese. Significant microbial activity may occur in accumulated sediments. Organic and inorganic particles can also accumulate in low-flow areas or dead-ends of the DWDS, and enhance microbial activity by providing protection and nutrients [177]. Biofilms that slough can accumulate in the periphery of distribution systems leading to sediment accumulation and the proliferation of some microorganisms [178]. Sediment accumulation may also lead to decrease of disinfectant residual in water. There are inorganic particles, like sand, that will promote the erosion of biofilm while others, like clay, may result in thicker and stronger biofilms [7,179].

## MECHANISMS OF BIOFILM FORMATION IN DRINKING WATER DISTRIBUTION SYSTEMS

The understanding of the mechanisms of microbial growth in DWDS like the microbial ecology, specific mechanisms of adhesion, intra and interspecies interactions and the production of signalling and other metabolite molecules, will continue to provide needed insights to help resolving public health concerns associated with biofilm formation on these systems. The standard methods of disinfection are not efficient in DWDS biofilm control [1]. Recent findings into the microbial ecology of distribution systems have found that pathogenic resistance to chlorination is affected by microbial community diversity and interspecies relationships [175].

### Microbial Community Diversity

A DWDS provides a habitat for microorganisms, which are sustained by organic and inorganic nutrients present on the pipe and in the conveyed water [180]. According to Berry *et al*. [175] an understanding of the microbial ecology of the distribution system is necessary to design innovative and effective control strategies that will ensure safe and high quality DW to the consumer.

In general, heterotrophic plate counts (HPC) are used to assess the overall bacterial quality of DW [181]. However, the majority of bacterial cells in natural communities are either non-cultivable by current cultivation methods or are present in a viable but non-cultivable (VBNC) state [182]. So, such methods are now known to significantly underestimate the total number of bacteria in DW [31]. Thus, the real composition and dynamics of bacterial communities in DWDS are far from being assessed and understood in detail.

The biodiversity of bacterial population in DW biofilms is still poorly understood, but biomolecular tools bring new light on population composition and dynamics [183-190]. Through these molecular approaches, *Proteobacteria*, particularly of the classes *α-proteobacteria, β-proteobacteria, γ-proteobacteria and δ-proteobacteria*, have been found to predominate in chlorinated DW [188,190-191].

The microbial composition of DWDS communities is influenced by several factors and reflects the microflora characteristics of the raw water source [190]. Previous research has shown that distribution system pipe material, temperature, the level of organic carbon available, velocity of water and the disinfectant used in a system are among the factors that may impact the growth and community structure of DWDS biofilms [106,108,121,188,192] According to Williams *et al.* [188], following exposure to either free chlorine and monochloramine, *α-proteobacteria* was the predominant phylogenetic group observed in the treated distribution water, suggesting that these organisms are well suited to survive in potable water supplies. Conversely, *β-proteobacteria* were found to be more abundant in chloraminated water than in chlorinated water. In another study, Emtiazi *et al.* [25] revealed that *β-proteobacteria* were also abundant in biofilms of non-chlorinated DW. These studies indicate that microbial community diversity is impacted by the disinfection strategy. There is also evidence that diversity can affect disinfection efficacy and pathogen survival [175]. Simões *et al.* [193] provide experimental evidences on the role of the microbial diversity of DW-isolated bacteria biofilms in their resistance to chlorine disinfection.

In DWDS, *Acinetobacter*, *Aeromonas*, *Alcaligenes*, *Arthrobacter*/*Corynebacterium*, *Bacillus*, *Burkholderia*, *Citrobacter*, *Enterobacter*, *Flavobacterium*, *Klebsiella*, *Methylobacterium*, *Moraxella*, *Pseudomonas*, *Serratia*, *Staphylococcus*, *Mycobacterium*, *Sphingomonas* and *Xanthomonas* have been the predominant bacterial genera detected [175,194]. The Gram-negative are predominant over the Gram-positive bacteria, and *Pseudomonas* species are the most abundant bacterial organism in supply systems, regardless the water source.

In chloraminated systems several authors detected ammonia- and nitrite-oxidazing bacteria such as, *Nitrosomonas* and *Nitrobacter* belonging to the *β-proteobacteria* and *α-proteobacteria*, respectively and the genus *Nitrospira* [22,189,195].

There are some published studies where no pathogens were detected in DWDS [84,159,186]. According to Payment and Robertson [180], most of the microorganisms developed in distribution network are harmless. However, this dominant non-pathogenic bacterial populations should not be neglected, since they play a major role in biofilm formation [27] and biofouling [196]. Also, the autochthonous microbial community may promote the survival and growth of hygienically relevant and potentially pathogenic bacteria [197]. Nevertheless, other published studies detected several pathogens in DWDS such as: potentially pathogenic mycobacteria were detected in water samples collected in France [198]; infectious enteroviruses and adenoviruses were detected in water samples in urban sites of Korea [199]; opportunistic pathogens, *Mycobacterium* sp., *Legionella* spp. and *P. aeruginosa* were detected in biofilms and DW in Germany [25]; *Helicobacter* spp. was identified in biofilms [200]; *Aeromonas* spp. have also been found in DWDS [201], and in Russia and Bulgaria some water samples were positive for *Giardia* and *Cryptosporidium* [202].

Additionally, the autochthonous microflora could sustain the growth of protozoa and metazoa that are visible or may have adverse effects on the taste of the DW [190]. Filamentous fungi and microfungi were also observed in DWDS [14,203-204].

## Microbial Interactions

Under natural conditions, true monospecies biofilms are rare and in most natural and industrial environments, such as DWDS, biofilms are complex communities. Diversity in microbial communities leads to a variety of complex relationships involving interspecies and intraspecies interactions. Interactions among bacterial species may have a profound influence on the initial stages of biofilm formation and development.

The ecology of a biofilm is a complex function of prevailing growth conditions, hydrodynamic forces, presence of microbial metabolites and molecules (cell-to-cell signalling communications) excreted by the microorganisms and dominant microbial inhabitants in the biofilm [47]. Bacteria have the ability to signal and sense the state of population density in order to change physiological needs under different growth conditions. This phenomenon is commonly called quorum-sensing (QS) [205]. Therefore, QS is a strategy of cell-to-cell communication benefiting the biofilm community by controlling unnecessary overpopulation and competition for nutrients [206]. QS has been demonstrated to play a role in cell attachment and detachment from biofilms [20,82]. Bacteria are considered colonial microorganisms by nature and exploit elaborate systems of intercellular interactions and communications to facilitate their adaptation to changing environments [8,207-209]. The successful adaptation of bacteria to changing natural conditions is dependent on their ability to sense and respond to the external environment and modulate gene expression accordingly [20].

Surfaces provide a niche that promotes the evolution of complex interactions between bacterial cells. Once cells are firmly bound, the activity of the community is dependent on the metabolism and growth of each member species under local surface conditions. Such metabolic activities can include substrate consumption, cellular growth and replication, and synthesis of extracellular polymeric substances [47]. The biological complexity of a system is defined by intra as well as interpopulation cell behaviour. The metabolic activities of those microorganisms that become associated with a surface cause these interfacial chemical gradients to evolve over time and space, creating conditions not normally encountered in the bulk aqueous phase [210].

The microbial heterogeneity found in DW and the existence of interspecies relationships can provide improved strategies for microbial growth control [193,211]. Competition for substrate is considered to be one of the major evolutionary driving forces in the microbial world, and experimental data obtained in laboratory conditions showed how different microorganisms may effectively outcompete others because of better utilization of a given energy source [16,212]. Central to the structure, composition and function of any community is a complex set of interactions. For instance, Hansen *et al.* [213] found that spatial structure was the key environmental factor for *P. putida* KT2440 and *Acinetobacter* sp. strain C6 to establish a structured community for interspecies interactions. Previously, Møller *et al.* [212]

showed the metabolic synergy between *P. putida* and *Acinetobacter* sp. community members when biodegrading toluene and related aromatic compounds. There is evidence that biofilm community diversity can affect disinfection efficacy and pathogen survival within biofilms [214].

Most of the research into interspecies interactions within biofilms has focused on the beneficial aspects of these relationships. However, not all interactions will be beneficial, since antagonistic interactions may play an important role in the development of microbial communities. The production of antimicrobial molecules, including toxins, bacteriolytic enzymes, antibiotics and bacteriocins seems to be a generic phenomenon for most bacteria [215-216]. Table 2 shows relevant interactions found for several multispecies biofilms from diverse environments.

**Table 2. Relevant interspecies interactions in biofilm communities**

| **Interspecies interactions** | **Strains** | **Reference** |
|---|---|---|
| Antagonism | Marine epiphytic bacteria | [237] |
| | Enteric bacteria | [216] |
| | Marine pelagic bacteria | [238] |
| | DW-isolated bacteria | [18] |
| | *Bacillus cereus*/*Pseudomonas fluorescens* | [239] |
| Commensalism | *Pseudomonas* sp. strain GJ1/*Pseudomonas putida* DMP1 | [240] |
| | *Acinetobacter* sp./*Pseudomonas putida* | [16] |
| | *Lactococcus lactis* ssp. *cremoris*/*Pseudomonas fluorescens* | [241] |
| Competition | *Acinetobacter* sp./*Pseudomonas putida* | [16] |
| | *Klebsiella oxytoca*/*Burkolderia cepacia* | [242] |
| | Marine epiphytic bacteria | [17] |
| | Denitrifying bacteria | [243] |

| | | |
|---|---|---|
| Mutualism (protocooperation and symbiose) | Soil bacteria | [244] |
| | Oral bacteria | [245] |
| | Marine epiphytic bacteria | [214] |
| | *E. coli* PHL565/ *P. putida* MT2; *E. coli* PHL565/environmental *E. coli* from DWDS | [246] |
| | DW-isolated bacteria | [18] |
| Neutralism | *Pseudomonas* sp.; *Corynebacterium* sp.; *Candida* sp.; *Schizosaccharomyces* sp.; *Saccharomyces* sp.; *Schizosaccharomyces* sp. | [247] |
| | DW-isolated bacteria | [18] |

## Coaggregation

Coaggregation, the specific recognition and adherence of genetically distinct bacteria to one another, occurs in a variety of ecosystems [217-219] and was first demonstrated for bacteria from dental plaque [220], where both intergeneric and intrageneric coaggregation occurs [221]. However, coaggregation is a widespread phenomenon that has now been observed amongst bacteria from other biofilm communities in several diverse habitats. Few reports on the coaggregation abilities of freshwater biofilm bacteria have been published [219,222-225], and it has been suggested that coaggregation may also mediate the sequential integration of species of bacteria into freshwater biofilms [226-227].

This mechanism of adhesion is highly specific and is thought to have a role in the development of multispecies biofilms in many different environments [221,228-231] and is now recognized as a mechanism for allowing specific association between collaborating bacterial species. Aggregation conveys advantages to microorganisms including transfer of chemical signals, exchange of genetic information, protection from adverse environmental conditions, metabolic cooperation between different species, as well as cell differentiation in some populations [232]. Coaggregation interactions contribute to the development of biofilms by two routes. The first route is by single cells in suspension specifically recognizing and adhering to genetically distinct cells in the developing biofilm. The second is by the prior coaggregation in suspension of secondary colonizers followed by the subsequent adhesion of this coaggregate to the developing biofilm [229]. In both cases, bacterial cells in suspension specifically adhere to biofilm cells in a process known as coadhesion [51,233].

Coaggregation between pairs of freshwater bacteria is typically mediated by protein “adhesion” on one cell type and a complementary saccharide “receptor” on the other. These protein-saccharide interactions can be blocked by the addition of simple sugars [222,229-230]. Thus, the mechanism mediating adhesion between coaggregating pairs in freshwater

biofilm bacteria is very similar to the one verified in oral bacteria. The coaggregation between freshwater bacteria is growth-phase-dependent. It depends on cells being in the optimum physiological state for coaggregation and it is maximized when both partner bacteria are in stationary phase. Maximum expression of coaggregation generates clearly visible flocs of cells in mixtures of the two types of cells [226] and is maintained for up to 48 h into stationary phase, depending on the coaggregating pair. The ability to coaggregate then decreases and eventually is lost completely [223]. The optimum coaggregation between a pair might be dependent upon a change in coaggregation ability of one or both partner bacteria. Moreover, and as suggested by Malik *et al.* [218], bacterial cell surface properties, namely the hydrophobicity, are other factor thought to play an important role in coaggregation, as well as in cell-substratum interactions. Recently, Min *et al.* [234] studied the influence of some physicochemical parameters (ionic strength, pH, temperature, and viscosity) on coaggregation ability between the freshwater bacteria. These physicochemical factors are important to consider when developing buffers to detect coaggregation in freshwater environments as well as when the intent is to develop novel approaches to control freshwater biofilm formation by blocking bacterial coaggregation.

Studies on freshwater biofilm bacteria have also demonstrated that coaggregation often occurs between bacteria that are taxonomically distant (intergeneric coaggregation) and occasionally between strains belonging to the same species (intraspecies coaggregation) [222,224]. Intergeneric coaggregation is common between oral bacteria [228], but intraspecies coaggregation has not yet been referred between oral plaque bacteria. Thus, intraspecies coaggregation may well be a characteristic that is unique to freshwater biofilm bacteria.

Simões *et al.* [230] investigated the intergeneric coaggregation ability among DW-isolated bacteria and the role of this specific mechanism in multispecies biofilm formation. This is the first report demonstrating that *A. calcoaceticus* has a bridging function in DW biofilm formation. This bacterium may facilitate the association of the other species that do not coaggregate directly with each other, increasing the opportunity for metabolic cooperation. Other report by Min and Rickard [231] also explores the role of coaggregation by freshwater bacteria in dual biofilm formation. These authors concluded that coaggregation promotes biofilm integration by facilitating attachment to partner species and likely contributes to the expansion of coaggregating *S. natatoria* populations in dual-species biofilms through competitive interactions. These studies raise the question of whether freshwater bridging organisms such as *A. calcoaceticus* and *S. natatoria* can aid the retention of microbial pathogens or if the ability to coaggregate with many species (i.e. bridge) is a mechanism to outcompete other species in freshwater multispecies biofilms.

Bacteria are affected by the environment they live in and the variety of other species present. Coaggregation can take the form of intra, inter or multigeneric interactions, a combination of which contributes to the overall structure and diversity of bacterial community in freshwater biofilms. The specific mechanism for this remains unknown, but a more complete picture of microbial community diversity and interspecies relationships should facilitate a better understanding of disinfection resistance phenomena and will provide new data to design innovative and effective control strategies that will guarantee microbial safe and high quality DW.

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## REFERENCES

[1] Simões, L. C. & Simões, M. (2013). Biofilms in drinking water: problems and solutions. *RSC Advances, 3*, 2520–2533.

[2] Simões, L. C. (2012). Biofilms in drinking water: formation and control. LAP LAMBERT Academic Publishing (ISBN 978-3848413508).

[3] Hall-Stoodley, L. & Stoodley, P. (2005). Biofilm formation and dispersal and the transmission of human pathogens. *Trends in Microbiology, 13*, 7-10.

[4] Huq, A., Whitehouse, C. A., Grim, C. J., Alam, M. & Colwell, R. R. (2008). Biofilms in water, its role and impact in human disease transmission. *Current Opinion in Biotechnology, 19*, 244–247.

[5] Costerton, J. W., Stewart, P. S. & Greenberg, E. P. (1999). Bacterial biofilms: a common cause of persistent infections. *Science, 284*, 1318-1322.

[6] Prakash, B., Veeregowda, B. M. & Krishnappa, G. (2003). Biofilms: A survival strategy of bacteria. *Current Science, 85*, 1299-1307.

[7] Vieira, M. J. & Melo, L. F. (1995). Effect of clay particles on the behaviour of biofilms formed by *Pseudomonas fluorescens*. *Water Science and Technology, 32*, 45-52.

[8] Simões, M., Simões, L. C. & Vieira, M. J. (2010b). A review of current and emergent biofilme control statagies. *LWT – Food Science and Technology, 43*, 573-583.

[9] Characklis, W. G. & Marshall, K. C. (1990). Biofilms: a basis for an interdisciplinary approach. In W. G. Characklis, & K. C. Marshall (Eds.), Biofilms. New York: John Wiley & Sons, Inc.

[10] Momba, M., Kfir, R., Venter, S. N. & Cloete, T. E. (2000). An overview of biofilm formation in distribution systems and its impact on the deterioration of water quality. *Water SA, 26*, 59–66.

[11] Codony, F., Miranda, A. M. & Mas, J. (2003). Persistence and proliferation of some unicellular algae in drinking water systems as result of their heterotrophic metabolism. *Water SA, 29*, 113-116.

[12] Skraber, S., Schijven, J., Gantzer, C. & de RodaHusman, A. M. (2005). Pathogenic viruses in drinking-water biofilms: a public health risk? *Biofilms, 2*, 105–117.

[13] Snelling, W. J., Moore, J. E., McKenna, J. P., Lecky, D. M. & Dooley, J. S. G. (2006). Bacterial-protozoa interactions: an update on the role these phenomena play towards human illness. *Microbes and Infection, 8*, 578-587.

[14] Siqueira, V. M., Oliveira, H. M., Santos, C., Paterson, R., Gusmao, N. & Lima, N. (2011). Filamentous Fungi in drinking water, particularly in relation to biofilm formation. *International Journal of Environmental Research and Public Health, 8*, 456-469.

[15] Nielsen, A. T., Tolker-Nielsen, T., Barken, K. B. & Molin, S. (2000). Role of commensal relationships on the spatial structure of a surface attached microbial consortium. *Environmental Microbiology, 2*, 59-68.

[16] Christensen, B.B., Haagensen, J. A. J., Heydorn, A. & Molin, S. (2002). Metabolic commensalism and competition in a two-species microbial consortium. *Applied and Environmental Microbiology, 68*, 2495-2502.

[17] Rao, D., Webb, J. S. & Kjelleberg, S. (2005). Competitive interactions in mixed species biofilms containing the marine bacterium *Pseudoalteromonas tunicata*. *Applied and Environmental Microbiology, 71*, 1729-1736.

[18] Simões, L. C., Simões, M. & Vieira, M. J. (2007b). Biofilm interactions between distinct bacterial genera isolated from drinking water. *Applied and Environmental Microbiology, 73*, 6192-6200.

[19] Watnick, P. & Kolter, R. (2000). Biofilm, city of microbes. *Journal of Bacteriology, 182*, 2675-2679.7

[20] Daniels, R., Vanderleyden, J. & Michiels, J. (2004). Quorum sensing and swarming migration in bacteria. *FEMS Microbiology Reviews, 28*, 261-289.

[21] Parsek, M. R. & Greenberg, E. P. (2005). Sociomicrobiology: the connections between quorum sensing and biofilms. *Trends in Microbiology, 13*, 27-33.

[22] Regan, J. M., Harrington, G. W., Baribeau, H., Leon, R. D. & Noguera, D. R. (2003). Diversity of nitrifying bacteria in full-scale chloraminated distribution systems. *Water Research, 37*, 197-205.

[23] Beech, I. B. & Sunner, J. (2004). Biocorrosion: towards understanding interactions between biofilms and metals. *Current Opinion in Biotechnology, 15*, 181-186.

[24] Camper, A. K. (2004). Involvement of humic substances in regrowth. *International Journal of Food Microbiology, 92*, 355-364.

[25] Emtiazi, F., Schwartz, T., Marten, S. M., Krolla-Sidenstein, P. & Obst, U. (2004). Investigation of natural biofilms formed during the production of drinking water from surface water embankment filtration. *Water Research, 38*, 1197-1206.

[26] Teng, F., Guan, Y. T. & Zhu, W. P. (2008). Effect of biofilm on cast iron pipe corrosion in drinking water distribution system: Corrosion scales characterization and microbial community structure investigation. *Corrosion Science, 50*, 2816-2823.

[27] LeChevallier, M. W., Babcock, T. M. & Lee, R. G. (1987). Examination and characterization of distribution system biofilms. *Applied and Environmental Microbiology, 53*, 2714-2724.

[28] Wingender, J. & Flemming, H.-C. (2011) Biofilms in drinking water and their role as reservoir for pathogens. *International Journal of Hygiene and Environmental Health, 214*, 417– 423.

[29] Flemming, H.-C., Percival, S. & Walker, J. (2002). Contamination potential of biofilms in water distribution systems. *Water Science and Technology: Water Supply, 2*, 271-280.

[30] Percival, S. L. & Walker, J. T. (1999). Potable water and biofilms: a review of the public health implications. *Biofouling, 42*, 99-115.

[31] Szewzyk, U., Szewzyk, R., Manz, W. & Schleifer, K. H. (2000). Microbiological safety of drinking water. *Annual Reviews in Microbiology, 54*, 81-127.

[32] September, S. M., Els, F. A., Venter, S. N. & Brözel, V. S. (2007). Prevalence of bacterial pathogens in biofilms of drinking water distribution systems. *Journal of Water and Health, 05.2*, 219-227.

[33] WHO (2011). Guidelines for drinking water quality (4th ed.). Geneva, Switzerland: World Health Organization.

[34] Tyndall, R. L. & Domingue, E. L. (1982). Co-cultivation of *Legionella pneumophila* and free-living amoebas. *Applied and Environmental Microbiology, 44*, 954-959.

[35] Thomas, J. M. & Ashbolt, N. J. (2011). Do free-living amoebae in treated drinking water systems present an emerging health risk? *Environmental Science and Technology, 45*, 860–869.

[36] Payment, P., Franco, E. & Siemiatycki, J. (1993). Absence of relationship between health effects due to tap water consumption and drinking water quality parameters. *Water Science and Technology, 27*, 137-143.

[37] Ford, T. E. (1999). Microbiological safety of drinking water: United States and global perspectives. *Environmental Health Perspectives, 107*, 191-206.

[38] Sharma, S., Sachdeva, P. & Virdi, J. S. (2003). Emerging waterborne pathogens. *Applied Microbiology and Biotechnology, 61*, 424-428.

[39] Hrudey, S. E. & Hrudey, E. J. (2004). Safe drinking water. Lessons from recent outbreaks in affluent nations. London: International Water Association Publishing.

[40] Craun, M. F., Craun, G. F., Calderon, R. L. & Beach, M. J. (2006). Waterborne outbreaks reported in the United States. *Journal of Water and Health, 4*, 19–30.

[41] Beaudeau, P., de Valk. H., Vaillant, V., Mannschott, C., Tillier, C., Mouly, D. & Ledrans, M. (2008). Lessons learned from ten investigations of waterborne gastroenteritis outbreaks, France, 1998–2006. *Journal of Water and Health, 6*, 491–503.

[42] Blasi, M. F., Carere, M., Pompa, M. G., Rizzuto, E. & Funari, E. (2008). Water-related diseases outbreaks reported in Italy. *Journal of Water and Health, 6*, 423–432.

[43] O'Toole, G., Kaplan, H. B. & Kolter, R. (2000). Biofilm formation as microbial development. *Annual Reviews in Microbiology, 54*, 49-79.

[44] Donlan, R. M. & Costerton, J. W. (2002). Biofilms: survival mechanisms of clinically relevant microorganisms. *Clinical Microbiology Reviews, 15*, 167-193.

[45] Hall-Stoodley, L. & Stoodley, P. (2002). Developmental regulation of microbial biofilms. *Current Opinion in Biotechnology, 13*, 228-233.

[46] Chmielewski, R.A.N. & Frank, J. F. (2003). Biofilm formation and control in food processing facilities. *Comprehensive Reviews in Food Science and Food Safety, 2*, 22-32.

[47] Bryers, J. D. & Ratner, B. D. (2004). Bioinspired implant materials befuddle bacteria. *ASM News, 70*, 232-237.

[48] Verstraeten, N., Braeken, K., Debkumari, B., Fauvart, M., Fransaer, J., Vermant, J. & Michiels, J. (2008). Living on a surface: swarming and biofilm formation. *Trends in Microbiology, 16*, 496-506.

[49] Simões, M., Bennett, R. N. & Rosa, E. A. S. (2009). Understanding antimicrobial activities of phytochemicals against multidrug resistant bacteria and biofilms. *Natural Product Reports, 26*, 746-757.

[50] Marshall, K. C. (1996). Adhesion as a strategy for access to nutrients. In M. Fletcher (Ed.), Bacterial adhesion: molecular and ecological diversity. Wiley Series.

[51] Busscher, H. J., Bos, R. & van der Mei, H. C. (1995). Initial microbial adhesion is a determinant for the strength of biofilm adhesion. *FEMS Microbiology Letters, 128*, 229–234.

[52] Bos, R., van der Mei, H. C. & Busscher, H. J. (1999). Physico-chemistry of initial microbial adhesive interactions - its mechanisms and methods for study. *FEMS Microbiology Reviews, 23*, 179-229.

[53] Freter, R., Gingell, D., Jones, G. W., Mutter, P. R. & Dazzo, F. B. (1984). Mechanisms of adhesion. In: K. C. Marshall (Ed.), Microbial adhesion and aggregation, (pp. 5-19). Berlin: Springer, Verlag.

[54] Oliveira, D. R. (1992) Physico-chemical aspects of adhesion. In L. F. Melo, T. R. Bott, M. Fletcher, & B. Capdeville (Eds.), Biofilms - science and technology (pp.45-58). Dordrecht: Kluwer Academic Publishers.

[55] Allison, D. G. & Sutherland, I. W. (1987). The role of exopolysaccharides in adhesion of freshwater bacteria. *Journal of Genetic Microbiology, 133*, 1319-1327.

[56] Watnick, P. & Kolter, R. (1999). Steps in the development of a *Vibrio cholerae* El Tor biofilm. *Molecular Microbiology, 34*, 586-95.

[57] Vieira, M. J., Melo, L. & Pinheiro, M. M. (1993). Biofilm formation: hydrodynamic effects on internal diffusion and structure. *Biofouling, 7*, 67-80.

[58] Bryers, J. D. (2000). Biofilm formation and persistence. In J. D. Bryers (Ed.), Biofilms II: Process analysis and applications, (pp. 45-88). New York: John Wiley & Sons.

[59] Characklis,W. G., Turakhia, M. H. & Zelver, N. (1990). Transport and interfacial transfer phenomena. In W. G. Characklis, & K. C. Marshall (Eds.), Biofilms. New York: John Wiley & Sons, Inc.

[60] Lee, S. F., Li, Y. H. & Bowden, G. W. (1996). Detachment of *Streptococcus mutans* biofilm cells by an endogenous enzymatic activity. *Infection and Immunity, 64*, 1035-1038.

[61] Allison, D. G., Ruiz, B., SanJose, C., Jaspe, A. Gilbert, P. (1998). Extracellular products as mediators of the formation and detachment of *Pseudomonas fluorescens* biofilms. *FEMS Microbiology Letters, 167*, 179-184.

[62] Wuertz, S., Okabe, S. & Hausner, M. (2004). Microbial communities and their interactions in biofilm systems: an overview. *Water Science and Technology, 49*, 327-336.

[63] Allison, D. G., Evans, D. J., Brown, M. R. W. & Gilbert, P. (1990). Possible involvement of the division cycle in dispersal of *Escherichia coli* from biofilms. *Journal of Bacteriology, 172*, 1667-1669.

[64] Gilbert, P., Evans, D. J. & Brown, M. R.W. (1993). Formation and dispersal of bacterial biofilms in vivo and in situ. *Journal of Appllied Bacteriology, 74*, 67S–78S.

[65] Rosenberg, E. & Ron, E. Z. (1999). High- and low-molecular-mass microbial surfactants. *Applied Microbiology and Biotechnology, 52*, 154-162.

[66] Picioreanu, C., van Loosdrecht, M. C. M. & Heijnen, J. J. (2001). Two dimensional model of biofilm detachment caused by internal stress from liquid flow. *Biotechnology Bioengineer, 72*, 205-218.

[67] Sauer, K., Cullen, M. C., Rickard, A. H., Zeef, L. A. H., Davies, D. G. & Gilbert, P. (2004). Characterization of nutrient-induced dispersion in Pseudomonas aeruginosa PAO1 biofilm. *Journal of Bacteriology, 186*, 7312-7326.

[68] Chen, X. & Stewart, P. S. (2002). Role of electrostatic interactions in cohesion of bacterial biofilms. *Applied Microbiology and Biotechnology, 59*, 718-720.

[69] Stewart, P. S. (1993). A model of biofilm detachment. *Biotechnology and Bioengineer, 41*, 111-117.

[70] Wimpenny, J. Manz, W. & Szewzyk, U. (2000). Heterogeneity in biofilms. *FEMS Microbiology Reviews, 24*, 661-671.

[71] Keevil, C. W. & Walker, J. T. (1992). Nomarski DIC microscopy and image analysis of biofilms. *Binary Computing Microbiology, 4*, 93-95.

[72] Lawrence, J. R., Korber, D. R., Hoyle, B. D., Costerton, J. W. & Caldwell, D. E. (1991). Optical sectioning of microbial biofilms. *Journal of Bacteriology, 173*, 6558-6567.

[73] Costerton, J. W., Lewandowski, Z., DeBeer, D., Caldwell, D., Korber, D. & James, G. (1994). Biofilms, the customized microniche. *Journal of Bacteriology, 176*, 2137-2142.

[74] Stoodley, P., DeBeer, D. & Lewandowski, Z. (1994). Liquid flow in biofilm systems. *Applied and Environmental Microbiology, 60*, 2711-2716.

[75] James, G. A., Korber, D. R., Caldwell, D. E. & Costerton, J W. (1995). Digital image analysis of growth and starvation responses of a surface- colonizing *Acinetobacter* sp. *Journal of Bacteriology, 177*, 907-915.

[76] Wimpenny, J. & Colasanti, R. (1997). A unifying hypothesis for the structure of microbial biofilms based on cellular automaton models. *FEMS Microbiology Ecology, 22*, 1-6.

[77] Wimpenny, J. (2000). An overview in biofilms as functional communities. In D. G. Allison, P. Gilbert, H. M. Lappin-Scott, & M. Wilson (eds.), Community structure and co-operation in biofilms (pp. 1-24). Cambridge, UK: Cambridge University Press.

[78] Wood, S. R., Kirkham, J., Marsh, P. D., Shore, R. C., Naltress, B. & Robinson, C. (2000). Architecture of intact natural human plaque biofilms studied by confocal laser scanning microscopy. *Journal of Dental Research, 79*, 21-27.

[79] Huang, C.-T., Xu, K. D., McFeters, G. A. & Stewart, P. S. (1998). Spatial patterns of alkaline phosphatase expression within bacterial colonies and biofilms in response to phosphate starvation. *Applied Environmental Microbiology, 64*, 1526-1531.

[80] Sutherland, I. W. (2001a). The biofilm matrix – an immobilized but dynamic microbial environment. *Trends in Microbiology, 9*, 222-227.

[81] Horn, H., Wasche, S. & Hempel, D. C. (2002). Simulation of biofilm growth, substrate conversion and mass transfer under different hydrodynamic conditions. *Water Science and Technology, 46*, 249-252.

[82] Donlan, R. M. (2002). Biofilms: microbial life on surfaces. *Emerging Infectious Diseases, 8*, 881-890.

[83] Chu, C. W., Lu, C. Y., Lee, C. M. & Tsai, C. (2003). Effects of chlorine level on the growth of biofilm in water pipes. *Journal of Environmental Science and Health Part A-Toxic/Hazardous Substances and Environmental Engineering, 38*, 1377-1388.

[84] Yu, S. L., Yu, X. J., Shi, W. X., Wang, D. & Qiu, X. X. (2007). Bacterial species and variation in the biofilm of water distribution system in Harbin city. *Journal of Water Supply Research and Technology-Aqua, 56*, 445-451.

[85] Lewandowski, Z. (2000). Structure and function of biofilms. In L. V. Evans (Ed.), Biofilms: recent advances in their study and control (pp.1-17). Amsterdam: Harwood Academic Publishers.

[86] Tolker-Nielsen, T. & Molin, S. (2000). Spatial organization of microbial biofilm communities. *Microbial Ecology, 40*, 75-84.

[87] Stoodley, P., Boyle, J. D., Dodds, I. & Lappin-Scott, H. M. (1997). Consensus model of biofilm structure. In J. W. T. Wimpenny, P. S. Handely, P. Gilbert, H. M. Lappin-Scott, & M. Jones (Eds.), Biofilms: community interactions and controls (pp. 1-9). Cardiff, UK: BioLine.

[88] Stoodley, P., Lewandowski, Z., Boyle, J. D. & Lappin-Scott, H. M. (1999**).** Influence of hydrodynamic conditions and nutrients on biofilm structure. *Journal of Applied Microbiology, 85*, 19S-28S.

[89] Sutherland, I. W. (2001b). Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology, 147*, 3-9.

[90] Stoodley, P., Sauer, K., Davies, D. G. & Costerton, J. W. (2002). Biofilms as complex differentiated communities. *Annual Reviews in Microbiology, 56*, 187-209.

[91] Costerton, J. W. (1995). Overview of microbial biofilms. *Journal of Industrial Microbiology & Biotechnology, 15*, 137-140.

[92] Costerton, J. W., Lewandowski, Z., Caldwell, D. E., Korber, D. R. & Lappin-Scott, H. M. (1995). Microbial biofilms. *Annual Review of Microbiology, 49*, 711-745.

[93] Zhang, X. Q., Bishop, P. L. & Kupferle, M. J. (1998). Measurements of polysaccharides and proteins in biofilm extracellular polymers. *Water Science and Technology, 37*, 345-348.

[94] Watnick, P. & Kolter, R. (2000). Biofilm, city of microbes. *Journal of Bacteriology, 182*, 2675-2679.7

[95] Flemming, H.-C., Wingender, J., Mayer, C., Körstgens, V. & Borchard, W. (2000). Cohesiveness in biofilm matrix polymers. In D. Allison, P. Gilbert, H. M. Lappin-Scott, & M. Wilson (Eds.), Community structure and cooperation in biofilms, (pp. 87-105). SGM Symposium 59: Cambridge University Press.

[96] Melo, L. F. (2003). Biofilm formation and its role in fixed film processes. In Elsevier (Ed.), The Handbook of Water and Wastewater Microbiology.

[97] Branda, S. S., Vik, A., Friedman, L. & Kolter, R. (2005). Biofilms: the matrix revisited. *Trends in Microbiology, 13*, 20-26.

[98] Stewart, P. S. & Costerton, J. W. (2001). Antibiotic resistance of bacteria in biofilms. *Lancet, 358*, 135-138.

[99] LeChevallier, M. W., Cawthon, C. D. & Lee, R. G. (1988a). Inactivation of biofilm bacteria. *Applied and Environmental Microbiology, 54*, 2492-2499.

[100] Hijnen, W. A. M., Beerendonk, E. F. & Medema, G. J. (2006). Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Research, 40*, 3-22.

[101] LeChevallier, M. W., Cawthon, C. D. & Lee, R. G. (1988b). Factors promoting survival of bacteria in chlorinated water supplies. *Applied and Environmental Microbiology, 54*, 649-654.

[102] Wolfaardt, G. M., Lawrence, J. R., Robarts, R. D. & Caldwell, D. E. (1998). In situ characterization of biofilm exopolymers involved in the accumulation of chlorinated organics. *Microbial Ecology, 35*, 213-223.

[103] Flemming, H.-C. (2002). Biofouling in water systems - cases, causes and countermeasures. *Applied Microbiology and Biotechnology, 59*, 629-640.

[104] Molin, S. & Tolker-Nielsen, T. (2003). Gene transfer occurs with enhanced efficiency in biofilms and induces enhanced stabilisation of the biofilm structure. *Current Opinion in Biotechnology, 14*, 255- 261.

[105] Lund, V. & Ormerod, K. (1995). The influence of disinfection processes on biofilm formation in water distribution systems. *Water Research, 29*, 1013-1021.

[106] Camper, A. K., Jones, W. L. & Hayes, J. T. (1996). Effect of growth condition and substratum composition on the persistence of coliforms in mixed-population biofilms. *Applied and Environmental Microbiology, 62*, 4014-4018.

[107] Volk, C. J. & LeChevallier, M. W. (1999). Impacts of the reduction of nutrient levels on bacterial water quality in distribution systems. *Applied and Environmental Microbiology, 65*, 4957–4966.

[108] Norton, C. D. & LeChevallier, M. W. (2000). A pilot study of bacteriological population changes through potable water treatment and distribution. *Applied and Environmental Microbiology, 66*, 268-276.

[109] Chu, C., Lu, C. & Lee, C. (2005). Effects of inorganic nutrients on the regrowth of heterotrophic bacteria in drinking water distribution systems. *Journal of Environmental Management, 74*, 255-263.

[110] Gagnon, G. A., Rand, J. L., O`Leary, K. C., Rygel, A. C., Chauret, C. & Andrews, R. C. (2005). Disinfectant efficacy of chlorite and chlorine dioxide in drinking water biofilms. *Water Research, 39*, 1809-1817.

[111] Lehtola, M. J., Laxander, M., Miettinen, I. T., Hirvonen, A., Vartiainen, T. & Martikainen, P. J. (2006). The effects of changing water flow velocity on the formation of biofilms and water quality in pilot distribution system consisting of copper or polyethylene pipes. *Water Research, 40*, 2151-2160.

[112] Lee, Y. & Nam, S. (2005). Comparative study of chemical disinfection in drinking water supplies. *Water Conditioning and Purification*, 1-6.

[113] LeChevallier, M. W., Welch, N. J. & Smith, D. B. (1996). Full-scale studies of factors related to coliform regrowth in drinking water. *Applied and Environmental Microbiology, 62*, 2201-2211.

[114] Momba, M. N. B., Cloete, T. E., Venter, S. N. & Kfir, R. (1998). Evaluation of the impact of disinfection processes on the formation of biofilms in potable surface water distribution systems. Water Science and Technology, 38, 283-289.

[115] Codony, F., Morato, J. & Mas, J. (2005). Role of discontinuous chlorination on microbial production by drinking water biofilms. Water Research, 39, 1986-1906.

[116] Chandy, J. P. & Angles, M. L. (2001). Determination of nutrients limiting biofilm formation and the subsequent impact on disinfectant decay. *Water Research*, 35, 2677-2682.

[117] Hallam, N. B., West, J. R., Forster, C. F., Powell, J. C. & Spencer, I. (2002). The decay of chlorine associated with the pipe wall in water distribution systems. *Water Research*, 36, 3479-3488.

[118] de Beer, D., Srinivasan, R. & Stewart, P. S. (1994). Direct measurement of chlorine penetration into biofilms during disinfection. *Applied and Environmental Microbiology, 60*, 4339-4344.

[119] Codony, F., Morato, J., Ribas, F. & Mas, J. D. (2002). Effect of chlorine, biodegradable dissolved organic carbon and suspended bacteria on biofilm development in drinking water systems. *Journal of Basic Microbiology, 42*, 311-319.

[120] Kokare, C. R., Chakraborty, S., Khopade, A. N. & Mahadik, K. R. (2009). Biofilm: importance and applications. *Indian Journal of Biotechnology, 8*, 159-168.

[121] 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. *Journal of Industrial Microbiology and Biotechnology, 22*, 152-159.

[122] 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*, 91-99.

[123] Paris, T., Skali-Lami, S. & Block, J. C. (2007). Effect of wall shear rate on biofilm deposition and grazing in drinking water flow chambers. *Biotechnology and Bioengineerin, 97*, 1550-1561.

[124] Soini, S. M., Koskinen, K. T., Vilenius, M. J. & Puhakka, J. A. (2002). Effects of fluid-flow velocity and quality on planktonic and sessile microbial growth in water hydraulic systems. *Water Research, 36*, 3812-3820.

[125] Chen, M. J., Zhang, Z. & Bott, T. R. (2005). Effects of operating conditions on the adhesive strength of *Pseudomonas fluorescens* biofilms in tubes. *Colloids and Surfaces B: Biointerfaces*, 43, 61-71.

[126] Tsai, Y. P. (2005). Impact of flow velocity on the dynamic behaviour of biofilm bacteria. *Biofouling, 21*, 267-277.

[127] Liu, Y. & Tay, J. H. (2001). Detachment forces and their influence on the structure and metabolic behaviour of biofilms. *World Journal of Microbiology & Biotechnology, 17*, 111-117.

[128] Liu, Y., Lin, Y. M., Yang, S. F. & Tay, J. H. (2003). A balanced model for biofilms developed at different growth and detachment forces. *Process Biochemistry, 38*, 1761-1765.

[129] van der Kooij, D. (1992). Assimilable organic carbon as an indicator of bacterial regrowth. *Journal American Water Works Association, 84*, 57–65.

[130] Owen, D. M., Amy, G. L., Chowdhury, Z. K., Paode, R., McCoy, G. & Viscosil, K. (1995). NOM characterization and treatability. *Journal American Water Works Association, 87*, 46–63.

[131] Servais, P., Laurent, P. & Randon, G. (1995). Comparison of the Bacterial Dynamics in Various French Distribution-Systems. *Journal of Water Supply Research and Technology-Aqua, 44*, 10-17.

[132] Batté, M., Koudjonou, B., Laurent, P., Mathieu, L., Coallier, J. & Prévost, M. (2003a). Biofilm responses to ageing and to a high phosphate load in a bench-scale drinking water system. *Water Research, 37*, 1351–1361.

[133] Servais, P., Anzil, A., Gatel, D. & Cavard, J. (2004). Biofilm in the Parisian suburbs drinking water distribution system. *Journal of Water Supply Research and Technology-Aqua, 53*, 313-324.

[134] LeChevallier, M. W., Schulz, W. & Lee, R. G. (1991). Bacterial nutrients in drinking water. *Applied and Environmental Microbiology, 57*, 857-862.

[135] Hu, J. Y., Yu, B., Feng, Y.Y., Tan, X. L., Ong, S. L., Ng, W. J. & Hoe, W. C. (2005). Investigation into biofilms in a local drinking water distribution system. *Biofilms, 2*, 19-25.

[136] Frias, J., Ribas, F. & Lucena, F. (2001). Effects of different nutrients on bacterial growth in a pilot distribution system. *Antonie Van Leeuwenhoek, 80*, 129-138.

[137] Bai, X. H., Zhang, L., Zhu, B., Jiang, C. T. & Wang, H. L. (2006). Relationship between water biological stability and the bacteria growth in drinking water distribution system. *Zhongguo Huanjing Kexue/China Environmental Science, 26*, 180-182.

[138] Sathasivan, A. & Ohgaki, S. (1999). Applicat.ion of new bacterial regrowth potential method for water distribution system - A clear evidence of phosphorus limitation. *Water Research, 33*, 137-144.

[139] Lehtola MJ, Miettinen IT, Vartiainen T and Martikainen PJ (2002) Changes in content of microbially available phosphorus, assimilable organic carbon and microbial growth potential during drinking water treatment processes. *Water Research* **36**: 3681-3690.

[140] Appenzeller, B. M. R., Batté, M., Mathieu, L., Block, J. C., Lahoussine, V., Cavard, J. & Gate, D. (2001). Effect of adding phosphate in drinking water on bacterial growth in slightly and highly corroded pipes. *Water Research, 35*, 1100–1105.

[141] Appenzeller, B. M. R., Duval, Y. B., Thomas, F. & Block, J. C. (2002). Influence of phosphate on bacterial adhesion onto iron oxyhydroxide in drinking water. *Environmental Science and Technology, 36*, 646-652.

[142] Veiga, M. C., Jain, M. K., Wu, W. M., Hollingsworth, R. I. & Zeikus, J. G. (1997). Composition and role of extracellular polymers in methanogenic granules. *Applied and Environmental Microbiology, 63*, 403-407.

[143] Wasche, S., Horn, H. & Hempel, D. C. (2002). Influence of growth conditions on biofilm development and mass transfer at the bulk/biofilm interface. *Water Research, 36*, 4775-4784.

[144] Zhang, T. C. & Bishop, P. L. (1994). Structure, Activity and Composition of Biofilms. *Water Science and Technology, 29*, 335-344.

[145] Melo, L. F. & Vieira, M. J. (1999). Physical stability and biological activity of biofilms under turbulent flow and low substrate concentration. *Bioprocess Engineering, 20*, 363-368.

[146] van Loosdrecht, M. C. M., Picioreanu, C. & Heijnen, J. J. (1997). A more unifying hypothesis for biofilm structures. *FEMS Microbiology Ecology, 24*, 181-183.

[147] van Loosdrecht, M. C. M,, Eikelboom, D., Gjaltema, A., Mulder, A., Tijhuis, L. & Heijnen, J. J. (1995). Biofilm structures. *Water Science and Technology, 32*, 35-43.

[148] Schwartz, T., Hoffmann, S. & Obst, U. (1998). Formation and bacterial composition of young, natural biofilms obtained from public bank-filtered drinking water systems. *Water Research, 32*, 2787-2797.

[149] Kerr, C. J., Osborn, K. S., Robson, G. D. & Handley, P. S. (1999). The relationship between pipe material and biofilm formation in a laboratory model system. *Journal of Applied Microbiology, 85*, 29S – 38S.

[150] Niquette, P., Servais, P. & Savoir, R. (2000). Impacts of pipe materials on densities of fixed bacterial biomass in a drinking water distribution system. *Water Research, 34*, 1952-1956.

[151] Cloete, T. E., Westaard , D. & van Vuuren, S. J. (2003). Dynamic response of biofilm to pipe surface and fluid velocity. *Water Science and Technology, 47*, 57-59.

[152] Yu, J., Kim, D. & Lee, T. (2010). Microbial diversity in biofilms on water distribution pipes of different materials. *Water Science Technology, 61*,163-171.

[153] Hem, L. J. & Skjevrak, I. (2002). Potential water quality deterioration of drinking water caused by leakage of organic compounds from materials in contact with the water. In Copenhagen: 20th NoDig Conference.

[154] Momba, M. N. B. & Kaleni, P. (2002). Regrowth and survival of indicator microorganisms on the surfaces of household containers used for the storage of drinking water in rural communities of South Africa. *Water Research, 36*, 3023-3028.

[155] Chang, Y. C., Puil, M. L., Biggerstaff, J., Randall, A. A., Schulte, A. & Taylor, J. S. (2003). Direct estimation of biofilm density on different pipe material coupons using a specific DNA-probe. *Molecular and Cellular Probes, 17*, 237–243.

[156] Bachmann, R. T. & Edyvean, R. G. J. (2006). AFM study of the colonisation of stainless steel by *Aquabacterium commune*. *International Biodeterioration & Biodegradation, 58*, 112-118.

[157] Pedersen, K. (1990). Biofilm development on stainless-steel and PVC surfaces in drinking-water. *Water Research, 24*, 239-243.

[158] Zacheus, O. M., Iivanainen, E. K., Nissinen, T. K., Lehtola, M. J. & Martikainen, P. J. (2000). Bacterial biofilm formation on polyvinyl chloride, polyethylene and stainless steel exposed to ozonated water. *Water Research, 34*, 63-70.

[159] Wingender, J. & Flemming, H.-C. (2004). Contamination potential of drinking water distribution network biofilms. *Water Science and Technology, 49*, 277-286.

[160] Lehtola, M. J., Miettinen, I. T., Keinanen, M. M., Kekki, T. K., Laine, O., Hirvonen, A., Vartiainen, T. & Martikainen, P. J. (2004). Microbiology, chemistry and biofilm development in a pilot drinking water distribution system with copper and plastic pipes. *Water Research, 38*, 3769-3779.

[161] van der Kooij, D., Vrouwenvelder, H. S. & Veenendaal, H. R. (1995). Kinetic aspects of biofilm formation on surfaces exposed to drinking water. *Water Science and Technology, 32*, 61-65.

[162] LeChevallier, M. W., Lowry, C. D., Lee, R. G. & Gibbon, D. L. (1993). Examining the Relationship between Iron Corrosion and the Disinfection of Biofilm Bacteria. *Journal American Water Works Association, 85*, 111-123.

[163] Butterfield, P. W., Camper, A. K., Biederman, J. A. & Bargmeyer, A. M. (2002a). Minimizing biofilm in the presence of iron oxides and humic substances. *Water Research, 36*, 3898-3910.

[164] Beech, I. B. (2004). Corrosion of technical materials in the presence of biofilms-current understanding and state-of-the art methods of study. *International Biodeterioration & Biodegradation, 53*, 177-183.

[165] Drenkard, E. & Ausubel, F. M. (2002). *Pseudomonas* biofilm formation and antibiotic resistance are linked to phenotypic variation. *Nature, 416*, 740-743.

[166] Harbron, R. S. & Kent, C. A. (1988). Aspects of cell adhesion. In L. F. Melo, T. R. Bott, & C. A. Bernardo (Eds.), (Vol. 145, pp. 125-140). NATO ASI series.

[167] Characklis, W. G. & Wilderer, P. A. (1989). Glossary. In W. G. Characklis, & P. A. Wilderer (Eds.), Structure and function of biofilms, (pp. 369-371). Chichester: John Wiley & Sons, Inc.

[168] Allison, D. G. (2003). The biofilm matrix. *Biofouling, 19*, 139-150.

[169] Jahn, A. & Nielsen, P. H. (1998). Cell biomass and exopolymer composition in sewer biofilms. *Water Science and Technology, 37*, 17-24.

[170] Wingender, J., Neu, T. R. & Flemming, H.-C. (1999). What are bacterial extracellular polymeric substances? In J. Wingender, T. R. Neu, & Flemming, H.-C. (Eds.) Microbial extracellular polymeric substances – characterization, structure and function (pp. 1-19). Berlin, Germany: Springer Berlin Heidelberg.

[171] Tsuneda, S., Aikawa, H., Hayashi, H., Yuasa, A. & Hirata, A. (2003). Extracellular polymeric substances responsible for bacterial adhesion onto solid surface. *FEMS Microbiology Letters, 223*, 287-292.

[172] Kalmbach, S., Manz, W. & Szewzyk, U. (1997). Dynamics of biofilm formation in drinking water: phylogenetic affiliation and metabolic potential of single cells assessed by formazan reduction and in situ hybridization. *FEMS Microbiology Ecology, 22*, 265–279.

[173] Sibille, I., Sime-Ngando, T., Mathieu, L. & Block, J. C. (1998). Protozoan bacterivory and *Escherichia coli* survival in drinking water distribution systems. *Applied and Environmental Microbiology, 64,* 197-202.

[174] Brown, M. R. W. & Barker, J. (1999). Unexplored reservoirs of pathogenic bacteria: protozoa and biofilms. *Trends in Microbiology, 7*, 46-50.

[175] Berry, D., Xi, C. & Raskin, L. (2006). Microbial ecology of drinking water distribution systems. *Current Opinion in Biotechnology, 17*, 297-302.

[176] Keevil, C. W. (2003). Rapid detection of biofilms and adherent pathogens using scanning confocal laser microscopy and episcopic differential interference contrast microscopy. *Water Science and Technology, 47*, 105-116.

[177] EPA. Health Risks from Microbial Growth and Biofilms in Drinking Water Distribution Systems. US Environmental Protection Agency Office of Ground Water and Drinking Water Standards and Risk Management Division 1200 Pennsylvania Ave., NW Washington DC; 2002.

[178] van der Kooij, D. (2000). The unified biofilm approach: a framework for addressing biological phenomena in distribution systems. International Distribution Research Symposium. Denver, CO.

[179] Melo, L. F. & Bott, T. R. (1997). Biofouling in water systems. *Experimental Thermal and Fluid Science, 14*, 375-381.

[180] Payment, P. & Robertson, W. (2004). The microbiology of piped distribution systems and public health. In R. Ainsworth (Ed.), Safe piped waste: managing microbial water quality in piped distribution systems. WHO Drinking-water Quality Series, London, UK: IWA Publishing.

[181] Sartory, D. P. (2004). Heterotrophic plate count monitoring of treated drinking water in the UK: a useful operational tool. *International Journal of Food Microbiology, 92*, 297–306.

[182] Oliver, J. D. (2000). The public health significance of viable but nonculturable bacteria. In R. R. Colwell, & D. J. Grimes (Eds.), Nonculturable microorganisms in the environment (pp. 277-300). Washington, DC: ASM Press.

[183] Kalmbach, S., Manz, W. & Szewzyk, U. (2000). Development of a new method to determine the metabolic potential of bacteria in drinking water biofilms, probe active counts (PAC). In H.-C. Flemming, U. Szewzyk, & T. Griebe (Eds), Biofilms, investigative methods and applications (pp. 107–121). Lancaster, UK: Technomic Publishing.

[184] Batté, M., Mathieu, L., Laurent, L. & Prévost, M. (2003b). Influence of phosphate and disinfection on the composition of biofilms from drinking water systems as measured by fluorescence in situ hybridization. *Canadian Journal of Microbiology, 49*, 741-753.

[185] Martiny, A. C., Jorgensen, T. M., Albrechtsen, H.-J., Arvin, E. & Molin, S. (2003). Long-term succession of structure and diversity of a biofilm formed in a model drinking water distribution system. *Applied and Environmental Microbiology, 69*, 6899–6907.

[186] Schmeisser, C., Stockigt, C., Raasch, C., Wingender, J., Timmis, K. N., Wenderoth, D. F., Flemming, H.-C., Liesegang, H., Schmitz, R. A., Jaeger, K.-E. & Streit, W. R. (2003). Metagenome survey of biofilms in drinking-water networks. *Applied and Environmental Microbiology, 69*, 7298–7309.

[187] Williams, M. M. & Braun-Howland, E. B. (2003). Growth of *Escherichia coli* in model distribution system biofilms exposed to hypochlorous acid or monochloramine. *Applied and Environmental Microbiology, 65*, 5463–5271.

[188] Williams, M. M., Santo Domingo, J. W., Meckes, M. C., Kelty, C. A. & Rochon, H. S. (2004). Phylogenetic diversity of drinking water bacteria in a distribution system simulator. *Journal of Applied Microbiology, 96*, 954-964.

[189] Hoefel, D., Monis, P. T., Grooby, W. L., Andrews, S. & Saint, C. P. (2005). Culture-independent techniques for rapid detection of bacteria associated with loss of chloramine residual in a drinking water system. *Applied and Environmental Microbiology, 71*, 6479-6488.

[190] Eichler, S., Christen, R., Holtje, C., Westphal, P., Botel, J., Brettar, I., Mehling, A. & Hofle, M. G. (2006). Composition and dynamics of bacterial communities of a drinking water supply system as assessed by RNA- and DNA-based 16S rRNA gene fingerprinting. *Applied and Environmental Microbiology, 72*, 1858-1872.

[191] Poitelon, J.-B., Joyeux, M., Welté, B., Duguet, J.-P., Prestel, E., Lespinet, O. & DuBow, M. S. (2009). Assessment of phylogenetic diversity of bacterial microflora in drinking water using serial analysis of ribosomal sequence tags. *Water Research, 43*, 4197-4206.

[192] Camper, A. K., Brastrup, K., Sandvig, A., Clement, J., Spencer, C. & Capuzzi, A. J. (2003). Effect of distribution system materials on bacterial regrowth. *Journal of American Water Works Association, 95*, 107-121.

[193] Simões, L. C., Simões, M. & Vieira, M. J. (2010a). Influence of the diversity of bacterial isolates from drinking water on resistance of biofilms to disinfection. *Applied and Environmental Microbiology, 76*, 6673-6679.

[194] Block, J.-C., Sibille, I., Gatel, D., Reasoner, D. J., Lykins, B. & Clark, R. M. (1997). Biodiversity in drinking water distribution systems: a brief review. In D. Sutcliffe (Ed.), The microbiological quality of water (pp. 63-71). London, UK: Freshwater Biological Association.

[195] Martiny, A. C., Albrechtsen, H.-J., Arvin, E. & Molin, S. (2005). Identification of bacteria in biofilm and bulk water samples from a nonchlorinated model drinking water distribution system: detection of a large nitrite-oxidizing population associated with *Nitrospira* spp. *Applied and Environmental Microbiology, 71*, 8611-8617.

[196] Bachmann, R. T. & Edyvean, R. G. J. (2005). Biofouling: an historic and contemporary review of its causes, consequences and control in drinking water distribution systems. *Biofilms, 2*, 197-227.

[197] LeChevallier, M. W. (1990). Coliform regrowth in drinking water: a review. *Journal of the American Water Works Association, 82*, 74–86.

[198] Le Dantec, C., Duguet, J. P., Montiel, A., Dumoutier, N., Dubrou, S. & Vincent, V. (2002). Chlorine disinfection of atypical *Mycobacteria* isolated from a water distribution system. *Applied and Environmental Microbiology, 68*, 1025-1032.

[199] Lee, S. H. & Kim, S. J. (2002). Detection of infectious enteroviruses and adenoviruses in tap water in urban areas in Korea. *Water Research, 36*, 248-256.

[200] Park, S. R., Mackay, W. G. & Reid, D. C. (2001). *Helicobacter* sp. recovered from drinking water biofilm sampled from a water distribution system. *Water Research, 35*, 1624-1626.

[201] Sen, K. & Rodgers, M. (2004). Distribution of six virulence factors in *Aeromonas* species isolated from US drinking water utilities: a PCR identification. *Journal of Applied Microbiology, 97*, 1077-1086.

[202] Karanis, P., Sotiriadou, I., Kartashev, V., Kourenti, C., Tsvetkova, N. & Stojanova, K. (2006). Occurrence of *Giardia* and *Cryptosporidium* in water supplies of Russia and Bulgaria. *Environmental Research, 102*, 260-271.

[203] Doggett, M. S. (2000). Characterization of fungal biofilms within a municipal water distribution system. *Applied and Environmental Microbiology, 66*, 1249-1251.

[204] Gonçalves, A. B., Paterson, R. R. M. & Lima, N. (2006). Survey and significance of filamentous fungi from tap water. *International Journal of Hygiene and Environmental Health, 209*, 257-264.

[205] Swift, S., Throup, J. P., Williams, P., Salmond, G. P. & Stewart, G. S. (1996). Quorum sensing: a population-density component in the determination of bacterial phenotype. *Trends in Biochemical Science, 21*, 214-219.

[206] Davey, M. E. & O`Toole, G. A. (2000). Microbial biofilms: from ecology to molecular genetics. *Microbiology and Molecular Biology Reviews, 64*, 847-867.

[207] Davies, D. G., Parsek, M. R., Pearson, J. P., Iglewski, B. H., Costerton, J. W. & Greenberg, E. P. (1998). The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science, 280*, 295-298.

[208] Sauer, K. & Camper, A. K. (2001). Characterization of phenotypic changes in *Pseudomonas putida* in response to surface-associated growth. *Journal of Bacteriology, 183*, 6579-6589.

[209] Fuqua, C. & Greenberg, E. P. (2002). Listening in on bacteria acyl-homoserine lactone signalling. *Nature Reviews in Molecular Cell Biology, 3*, 685-695.

[210] Geesey, G. G. (2001). Bacterial behaviour at surfaces. *Current Opinion in Microbiology, 4*, 296-300.

[211] Rasmussen, T. B., Skindersoe, M. E., Bjarnsholt, T., Phipps, R. K., Christensen, K. B., Jensen, P. O., Anderson, J. B., Koch, B., Larsen, T. O., Hentzer, M., Eberl, L., Høiby, N. & Givskov, M. (2005). Identity and effects of quorum-sensing inhibitors produced by *Penicilium* species. *Microbiology, 151*, 1325-1340.

[212] Møller, S., Steinberg, C., Andersen, J. B., Christensen, B. B., Ramos, J. L., Givskov, M. & Molin, S. (1998). In situ gene expression in mixed-culture biofilms: evidence of metabolic interactions between community members. *Applied and Environmental Microbiology, 64*, 721-732.

[213] Hansen, S. K., Rainey, P. B., Haagensen, J. A. J. & Molin, S. (2007). Evolution of species interactions in a biofilm community. *Nature, 445*, 533-536.

[214] Burmølle, M., Webb, J. S., Rao, D., Hansen, L. H., Sørensen, S. J. & Kjelleberg, S. (2006). Enhanced biofilm formation and increased resistance to antimicrobial agents

and bacterial invasion are caused by synergistic interactions in multispecies biofilms. *Applied and Environmental Microbiology, 72*, 3916-3923.

[215] Riley, M. A. (1998). Molecular mechanism of bacteriocin evolution. *Annual Review of Genetics, 32*, 255-278.

[216] Tait, K. & Sutherland, I. W. (2002). Antagonistic interactions amongst bacteriocin-producing enteric bacteria in dual species biofilms. *Journal of Applied Microbiology, 93*, 345-352.

[217] Kolenbrander, P. E. (2000). Oral microbial communities: biofilms, interactions, and genetic systems. *Annual Review of Microbiology, 54*, 413–437.

[218] Malik, A., Sakamoto, M., Hanazaki, S., Osawa, M., Suzuki, T., Tochigi, M. & Kakii, K. (2003). Coaggregation among nonflocculating bacteria isolated from activated sludge. *Applied and Environmental Microbiology, 69*, 6056–6063.

[219] Rickard, A. H., McBain, A. J., Ledder, R. G., Handley, P. S. & Gilbert, P. (2003a). Coaggregation between freshwater bacteria within biofilm and planktonic communities. *FEMS Microbiology Letters, 220*, 133–140.

[220] Gibbons, R J. & Nygaard, M. (1970). Interbacterial aggregation of plaque bacteria. *Archives of Oral Biology, 15*, 1397-1400.

[221] Kolenbrander, P. E., Andersen, R. N., Clemans, D. L., Whittaker, C. J. & Klier, C. M. (1999). Potential role of functionally similar coaggregation mediators in bacterial succession. In H. N. Newman, & M. Wilson (Eds.), Dental plaque revisited: oral biofilms in health and disease (pp. 171-186). Cardiff, UK: Bioline.

[222] Buswell, C. M., Herlihy, Y. M., Marsh, P. D., Keevil, C. W. & Leach, S. A. (1997). Coaggregation amongst aquatic biofilm bacteria. *Journal of Applied Microbiology, 83*, 477-484.

[223] Rickard, A. H., Stephen, A. L., Buswell, C. M., High, N. J. & Handley, P. S. (2000). Coaggregation between aquatic bacteria is mediated by specific-growth-dependent lectin-saccharide interations. *Applied and Environmental Microbiology, 66*, 431-434.

[224] Rickard, A. H., Leach, S. A., Hall, L. S., Buswell, C. M., High, N. J. & Handley, P. S. (2002). Phylogenetic relationships and coaggregation ability of freshwater biofilm bacteria. *Applied and Environmental Microbiology, 68*, 3644–3650.

[225] Rickard, A. H., Gilbert, P. & Handley, P. S. (2004). Influence of growth environment on coaggregation between freshwater biofilm bacteria. *Journal of Applied Microbiology, 96*, 1367-1373.

[226] Rickard, A. H., Thomas, J., Leach, S. A., Buswell, C. M., High, N. J. & Handley, P. S. (1999). Coaggregation amongst aquatic and oral bacteria is mediated by lectin-saccharide interactions. In J. Wimpenny, P. Gilbert, J. Walker, M. Brading, & R. Bayston (Eds.), Biofilms: the good, the bad and the ugly (pp. 343-354). Cardiff, UK: Bioline.

[227] Handley, P. S., Rickard, A. H., Leach, S. A., Buswell, C. M. & High, N. J. (2001). Coaggregation-is it a universal phenomenon? In P. Gilbert, D. Allison, M. Brading, J. Verran, & J. Walker (Eds.), Biofilm community interactions: chance or necessity? (pp. 1–10). Cardiff, UK: Bioline.

[228] Kolenbrander, P. E. & London, J. (1993). Adhere today, here tomorrow: oral bacterial adherence. *Journal of Bacteriology, 175*, 3247–3252.

[229] Rickard, A. H., Gilbert, P., High, N. J., Kolenbrander, P. E. & Handley, P. S. (2003b). Bacterial coaggregation: an integral process in the development of multispecies biofilms. *Trends in Microbiology, 11*, 94–100.

[230] Simões, L. C., Simões, M. & Vieira, M. J. (2008a). Intergeneric coaggregation between drinking water bacteria: evidence for the role *Acinetobacter calcoaceticus* as a bridging bacterium. *Applied and Environmental Microbiology, 74*, 1259-1263.

[231] Min, K. R. & Rickard, A. H. (2009). Coaggregation by the freshwater bacterium *Sphingomonas natatoria* Alters Dual-Species Biofilm Formation. *Applied and Environmental Microbiology, 75*, 3987-3997.

[232] Wimpenny, J. & Colasanti, R. (2004). A simple cellular automaton model for coaggregation. *Biofilms, 1*, 369-375.

[233] Bos, R., van der Mei, H. C., Meinders, J. M. & Busscher, H. J. (1994). Quantitative method to study co-adhesion of microorganisms in a parallel plate-flow chamber – basic principles of the analysis. *Journal of Microbiological Methods, 20*, 289–305.

[234] Min, K. R. Zimmer, M. N. & Rickard, A. H. (2010). Physicochemical parameters influencing coaggregation between the freshwater bacteria *Sphingomonas natatoria* 2.1 and *Micrococcus luteus* 2.13. *Biofouling, 26*, 931–940.

[235] Ashbolt, N. J. (2004). Microbial contamination of drinking water and disease outcomes in developing regions. *Toxicology, 198*, 229-238.

[236] Karanis, P., Kourenti, C. & Smith, H. (2007). Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. *Journal of Water and Health, 5*, 1-38.

[237] Burgess, J. G., Jordan, E. M., Bregu, M., Mearns-Spragg, A. & Boyd, K. G. (1999). Microbial antagonism: a neglected avenue of natural products research. *Journal of Biotechnology, 70*, 27-32.

[238] Bhattarai, H. D., Lee, Y. K., Cho, K. H., Lee, H. K. & Shin, H. W. (2006). The study of antagonistic interactions among pelagic bacteria: a promising way to coin environmental friendly antifouling compounds. *Hydrobiology, 568*, 417-423.

[239] Simões, M., Simões, L. C., Pereira, M. O. & Vieira, M. J. (2008c). Antagonism between *Bacillus cereus* and *Pseudomonas fluorescens* in planktonic systems and in biofilms. *Biofouling, 24*, 339-349.

[240] Cowan, S. E., Gilbert, E., Liepmann, D. & Keasling, J. D. (2000). Commensal interactions in a dual-species biofilm exposed to mixed organic compounds. *Applied and Environmental Microbiology, 66*, 4481-4485.

[241] Kives, J., Guadarrana, D., Orgaz, B., Rivera-Sen, A., Vazquez, J. & SanJose, C. (2005). Interactions in biofilms of *Lactococcus lactis* spp. *cremoris* and *Pseudomonas fluorescens* cultured in cold UHT milk. *Journal of Dairy Science, 88*, 4165-4171.

[242] Komlos, J., Cunningham, A. B., Camper, A. K. & Sharp, R. R. (2005). Interaction of *Klebsiella oxytoca* and *Burkholderia cepacia* in dual-species batch cultures and biofilms as a function of growth rate and substrate concentration. *Microbial Ecology, 49*, 114–125.

[243] Andersson, S., Dalhammar, G. & Rajarao, G. K. (2009). Persistence and competition of denitrifying bacteria in a dual-strain biofilm subjected to a natural wastewater flora. *Vatten, 65*, 87-92.

[244] Wolfaardt, G. M., Lawrence, J. R., Robarts, R. D., Caldwell, S. J. & Caldwell, D. E. (1994). Multicellular organization in a degradative biofilm community. *Applied and Environmental Microbiology*, *60*, 434-446.

[245] Palmer, R. J., Karmerzak, K., Hansen, M. C. & Kolenbrander, P. E. (2001). Mutualism versus independence: strategies of mixed-species oral biofilms in vitro using saliva as the sole nutrient source. *Infection and Immunity*, *69*, 5794-5804.

[246] Castonguay, M.-H., van der Schaaf, S., Koester, W., Krooneman, J., van der Meer, W., Harmsen, H. & Landini, P. (2006). Biofilm formation by *Escherichia coli* is stimulated by synergistic interactions and co-adhesion mechanisms with adherence-proficient bacteria. *Research in Microbiology*, *157*, 471-478.

[247] Yu, A., Vinarov, Z., Robysheva, N., Smirnov, V. N. & Sokolov, D. P. (2002). Studies of the stability of microbial association use in industrial biofiltering of gaseous discharges. *Applied Biochemistry and Microbiology*, *38*, 445-449.

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