7

Biofilms Impact on Drinking Water Quality

Anca Farkas[1], Dorin Ciatarâş[1] and Brânduşa Bocoş[2]
[1]*Someş Water Company,*
[2]*National Public Health Institute, Regional Public Health Center of Cluj,*
Cluj-Napoca,
Romania

1. Introduction

A paradigm shift currently occurs in microbiology, with significant impacts in a variety of environmental, medical and industrial applications. The old misconception of free floating microbes is invalidated by a different knowledge pattern: the great majority of terrestrial microorganisms live in communities associated to surfaces, called biofilms (Costerton et al, 1987; Flemming, 2008; Muntean, 2009). This organisation mode is associated to all surfaces in contact with water in drinking water processing, storage and distribution. Such biofilms are represented by structured consortia of sessile microorganisms characterized by surface attachment, self-produced exopolymeric matrix, structural, functional and metabolic heterogeneity, capable of intercellular communication by quorum-sensing and plurispecific composition.

Biofouling in drinking and industrial water systems has detrimental effects such as microbiological and chemical deterioration in water quality, corrosion inducing, drinking water treatment yield loss, efficiency reducing in cooling and heating exchange and transport, as well as in membrane processes (White et al, 1999; Flemming et al, 2002; LeChevallier and Au, 2004; Coetser and Cloete, 2005).

Biofilms are playing a major role in drinking and waste water treatment processes due to their enhanced properties of mineralization, bioaccumulation and bioadsorbtion. Despite the beneficial effects of the biological filter known as *schmutzdecke* in slow sand filtration or of the bio-sand filters, biofilms occurrence in other treatment stages, in drinking water networks and reservoirs represents a continuous challenge to water professionals. Drinking water associated biofilms induce residual disinfectants depletion and may cause aesthetic problems consisting in colour, odour and taste degradation due to chemical compounds released and more important, they pose a threat to human and animal health by hosting pathogenic or toxins producing bacteria, viruses, protozoa, algae, fungi and invertebrates. The great majority of water related health problems are the result of microbial contamination (Riley et al, 2011). Considering these aspects, naturally occurring biofilms in contact with drinking water were identified and described as microbial reservoirs for further contamination (Szewzyk et al, 2000; Wingender and Flemming, 2011).

The complex structure of drinking water associated biofilms is influenced by the microbial composition of source water and sediments (LeChevallier et al, 1987; Szewzyk et al, 2000;

Emtiazi et al, 2004). They may enter the distribution network, escaping the treatment and disinfection processes (known as breakthrough) and multiply in bulk water or biofilms. The two modes of multiplication are defined as regrowth (recovery of disinfectant injured cells) and aftergrowth (microbial growth in a distribution system) processes (Characklis, 1988; van der Kooij, 2003). Pathogenic microorganisms of concern may also emerge in drinking water systems by intrusion, due to external contamination events in different steps of water treatment, storage and transportation: cross connections, backflow events, pipe breaks, negative pressure and because of improper flushing and disinfection procedures.

2. Drinking water biofilms, emerging pathogens and opportunistic pathogens

The most alarming consequences as a result of biofouling in drinking water distribution systems consist in the presence, multiplication and dispersion into water of bacterial pathogens, opportunistic pathogens, parasitic protozoa, viruses and toxins releasing fungi and algae. They may appear as primary colonizers promoting the adhesion at the interface and subsequent biofilm formation (Costerton, 1994), but more often as secondary colonizers in ecological microniches offered by the existent attached community.

Emerging pathogens are those that have appeared in a human population for the first time, or have occurred previously but are increasing in incidence or expanding to areas where they have not previously been reported, usually over the last 20 years. They include: bacteria (pathogenic *E. coli*, *Helicobacter pylori*, *Campylobacter jejuni*, *Mycobacterium* avium complex), parasitic protozoa (*Cryptosporidium* spp., *Cyclospora cayetanensis*, *Toxoplasma gonidii*), viruses (noroviruses, hepatitis E) and toxic cyanobacteria (Hunter et al, 2003). Opportunistic pathogens are commonly members of water microbiota that would be normally harmless to a healthy individual but can infect a compromised host (US EPA, 2002).

Such microorganisms were detected worldwide in drinking water and associated biofilms and in raw water and sediments. In this context, establishing health-based targets, drinking water quality assurance implies effective preventive measures, such as sources water protection in order to reduce contamination risk in these strategic environments, as well as corrective actions stipulated in water safety plans prepared by water suppliers.

When cases of illness are registered, epidemiological studies are conducted in order to demonstrate similarities in genetic profiles of strains isolated from clinical and the environmental specimens, to track the source of infection. Drinking water and associated biofilms are often among the prime candidates tested when gastrointestinal diseases and different types of infections are recorded.

Developing countries are facing a major lack of safe drinking water, the transmission via faecal-oral route causing enormous numbers of severe water related illness and cases of deaths, especially in infants (Riley et al, 2011). Microbial source tracking approach is particularly important for drinking water sources, in order to identify the origin of contamination. Increasing population's access to clean drinking water and sanitation facilities is one of the first priorities of local and global authorities.

Even if the access to high quality drinking water is provided, neither humans in rich countries have definitely won the fight against microbes, yet. Tap water quality assurance is facing new challenges, consisting mainly in biofouling issues, emergent waterborne

pathogens, toxins releasing and opportunistic pathogens occurrence. Besides households, different branches of industry, especially food and pharmaceutical, health care facilities, schools, nursing homes and other critical areas are carefully included in monitoring assays. Recently is seriously considered the ability of some species, referred as opportunistic pathogens, to induce disease under certain circumstances in immunocompromised individuals: immunosuppressed, malnourished, diabetic, burn, cancer, AIDS, on haemodialysis, respiratory, with organ transplants patients (Rusin et al, 1997; Payment and Robertson, 2004). Sensitive subpopulations such as young children, elderly persons or pregnant women are also vulnerable to infections caused by opportunistic pathogens (Reynolds et al, 2008). Other special categories of exposed subjects consist in patients with indwelling cannulae and catheters, implant devices and contact lenses wearers. Opportunistic pathogens are becoming a major issue, causing from allergy or superficial infections to life-threatening systemic infections, since the ascending trend in congenital and acquired immunodeficiency affecting global population. Such species presence is often investigated, in addition to routine monitoring, within drinking water and associated biofilms, as wide-occurring bacteria of concern in the continuous increasing category of hospitalised and ambulatory immunocompromised persons (Glasmacher et al, 2003).

Even in drinking water carefully treated and distributed at high standards, pathogenic contamination and disease outbreaks may occur (Szewzyk et al, 2000; Wingender & Flemming, 2011) demonstrating the imperative requirement for comprehensive water safety plans implementation.

2.1 Drinking water quality assessment – Microbiological aspects and biofilms

Tap water supposes not to be and is not sterile, microbial load in bulk water consisting mainly in inoffensive heterotrophs, presumably coming from associated biofilms by detachment during dispersion. Routine monitoring of raw water sources, finishing water at the exit from treatment plant, drinking water in pipe networks, service reservoirs and finally at the consumers implies periodically investigations of a number of water samples collected with a frequency depending on the population deserved. According to European regulations, microbial indicators assessed by standardised conventional culturing techniques are: colony count at 37ºC, colony count at 22ºC, total coliforms, *Escherichia coli*, intestinal enterococci and *Clostridium perfringens*. The greatest microbial risk being associated with ingestion of water contaminated with human or animal faeces, thus the potential presence of pathogenic bacteria, viruses and cysts of protozoan parasites; faecal indices (*E. coli*, intestinal enterococci and *C. perfringens*) presence is routinely investigated.

The shortcomings of water quality monitoring based on faecal indicators and heterotrophic plate count, resulting in underestimation of drinking water microbial populations in numbers and composition are discussed worldwide considering the following:

- Only a small volume approximated to represent from 2×10^{-7} to 5×10^{-7}% of delivered drinking water is examined in routine monitoring (Allen, 2011);
- In drinking water systems, the high majority of bacteria, estimated at 95%, are located attached at the surfaces, while only 5% are found in water phase and detected by sampling as commonly used for quality control (Flemming et al, 2002). Other studies

are indicating bacterial numbers characterizing the biomass in pipe biofilms being 25 times more abundant than the suspended cells (Servais et al, 2004). Although, the common notion that biofilms dominate the distribution systems has been proven to be not true under all conditions by Srinivasan (2008), whose findings suggest that bulk bacteria may dominate in network sections containing chlorine residuals lower than 0.1mg/L and having residence time longer than 12 hours.

- A significant percent of water and biofilm bioburden may be in a viable but non cultivable state, unable to grow on artificial growth media but alive and capable of renewed activity and so hygienically relevant (Moritz et al, 2010). A small fraction of waterborne microorganisms (0.01%) are estimated to be culturable heterotrophic bacteria (Watkins and Jian, 1997; Exner et al, 2003);
- Limitations of detection methods (Lehtola et al, 2006; 2007; September et al, 2007). The investigation of drinking water associated biofilms from four European countries (France, Great Britain, Portugal and Latvia) confirmed *E. coli* presence by culturing techniques in one out of five pipes whereas all networks except one were positive for *E. coli* using the PNA FISH methods; their viability was also demonstrated. *E.coli* contributed with percents from 0.001% to 0.1% in the total bacterial numbers (Juhna et al., 2007);
- Faecal indicators are the best predictors of potential risks, but their concentrations rarely correlate perfectly with those of pathogens (Payment and Locas, 2011). Although in freshwater significant correlations have been established between faecal indices and pathogenic species, their presence in drinking water showed limited or no correlation with different species of pathogenic or opportunistic pathogenic bacteria, viruses, protozoa and fungi. Water quality assessment based only on the investigation of faecal indicators' presence proved to be insufficient when many waterborne outbreaks emerged. Still, until more reliable indices and methods of detection will be wider implemented, the well- known standardised procedures are applied in routine monitoring across the globe.

Many studies targeting attached microbial communities have been performed for quantification of the total number of germs, by different methods. They offer an unspecific overview upon microbial load, bringing certain information about drinking water treatment process efficiency and distribution system integrity. Still, the real composition and dynamics of microbial populations within drinking water associated biofilms represents a continuous challenge. Experimental biofilm succession monitored for a long term development indicated a stable population state after 500 days in a model drinking water distribution system. A homogenous composition of the population in the mature biofilm could mask a dynamic situation at a smaller scale (Martiny et al., 2003). Quantitative and prescriptive evaluation is the next target of scientific community. Prediction of microorganisms' behaviour in the distribution system water and biofilms requires greater understanding of the effects in microbial attachment, detachment, survival, multiplication and viability of three groups of abiotic and biotic factors: substratum physicochemical properties (type of materials), biofilm composition (microbial intra- and interspecific interactions) and bulk water characteristics (disinfectants residuals, oxygen and nutrients concentrations, system hydraulics, temperature).

2.1.1 Occurrence of bacteria in drinking water and associated biofilms

Among the nuisance bacteria regularly found in drinking water and biofilms, species that are not characteristic to the water environment may appear due to contamination events, with major impacts upon human health. Enteric bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *K. oxytoca*, *Enterobacter cloacae*, *E. aglomerans*, *Helicobacter pylori, Campylobacter* spp., *Shigella* spp., *Salmonella* spp., *Clostridium perfringens*, *Enterococcus faecalis*, *E. faecium*, as well as environmental bacteria becoming opportunistic pathogens *Legionella pneumophila*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Aeromonas hydrophila*, *A. caviae*, *Mycobacterium avium*, *M. xenopi*, together with other waterborne agents have been indicated to live in ecological microniches offered by drinking water associated biofilms (table 1).

When compared with planktonic counterparts, biofilm bacteria and other inhabitants display superior characteristics due to specialization within this emergent structure and to complex relationships established (Costerton, 1994). Community belonging, from a microbial perspective represents a benefit materialized in increasing the chances of survival in this oligotrophic environment by offering ecological microniches, establishing intra- and interspecific cooperation relationships by communication via quorum sensing and perpetuating individuals' resistance to disinfection agents. Even species not able to survive and most of them incapable of growth and multiplication in water were identified in associated biofilms; recent studies have demonstrated their ability to grow in those microniches. For example, *Legionella pneumophila* survives but does not multiply in sterile drinking water, its proliferation being dependent on parasitic relationship with other microorganisms: 14 species of amoebae, two species of ciliated protozoa, and one slime mould - *L. pneumophila* being described as protozoonotic bacteria (Murga et al, 2001; Fields et al, 2002; Declerck, 2010). In many outbreaks, the presence of pathogenic bacteria was not detected by routine monitoring, the correlation with faecal indicators found in tap water samples being defective. *E. coli* bacillus, the most popular faecal indicator, was chosen inter alia based on its incapacity of growth in water. Recent studies have shown its ability to multiplicate in drinking water associated biofilms under strictly anaerobic conditions (Latimer et al., 2010), so the indicative value of the faecal index of choice becomes questionable.

One of the advantages offered by drinking water biofilm organization to its members is represented by the enhanced resistance to disinfection residuals. The four hypothetical mechanisms of biofilm resistance involve slow antimicrobial penetration, deployment of adaptative stress responses, physiological heterogeneity in biofilm population and the presence of phenotypic variants or persister cells (Chambless et al, 2005). Another benefit from the microbial perspective consists in the emergence of genetically encoded resistance to biocides and antibiotics, and the spread of antimicrobial resistance genes in bacterial populations via mobile genetic elements, by lateral gene transfer. Integrons are genetic elements possessing a site-specific recombination system for assembling of resistance genes in gene cassettes. They play a major role in the rapid spread of antibiotic resistance in clinical environments. Gene cassettes encoding resistance to quaternary ammonium compounds (*qac*) and integron-integrase (*intIl*) genes characteristics for class 1 integron were recently recovered from environmental samples, including biofilms from a groundwater treatment plant (Gillings et al, 2009). The proximity of individuals in biofilm consortia and the extremely short generation times in bacteria multiplication are prerequisites for intensive rates of lateral gene transfer and thus resistance spreading and perpetuation in diverse natural or artificial ecosystems.

Bacteria	Samples type/Origin	Country	References
Escherichia coli	Biofilm - WDS	USA	LeChevallier et al, 1987
	Biofilm - WDS	Germany	Schmeisser et al, 2003
	Biofilm – WDS	France, England, Portugal, Latvia	Juhna et al, 2007
	Biofilm, Water - WDS	South Africa	September et al, 2007
	Biofilm - DWTP	Romania	Farkas et al, 2011
Faecal enterococci	Water - WDS	Korea	Lee et al, 2006
	Water - WDS	South Africa	September et al, 2007
	Biofilm - WDS	Portugal	Menaia et al, 2008
	Biofilm - DWTP	Romania	Farkas et al, 2011
Clostridium spp.	Biofilm - WDS	Portugal	Menaia et al, 2008
	Water - WDS	Greece	Kormas et al, 2010
	Biofilm - DTP	Romania	Farkas et al, 2011
Klebsiella spp.	Biofilm - WDS	USA	LeChevallier et al, 1987
	Water - WDS	Korea	Lee et al, 2006
	Biofilm, Water - WDS	South Africa	September et al, 2007
Pseudomonas spp.	Biofilm - WDS	Germany	Schmeisser et al, 2003
	Biofilm - WDS	Germany	Emtiazi et al, 2004
	Water - WDS	Korea	Lee et al, 2006
	Biofilm, Water - WDS	South Africa	September et al, 2007
	Biofilm - WDS	Portugal	Menaia et al, 2008
	Biofilm - DWTP	Romania	Farkas et al, 2011
Aeromonas spp.	Water - WDS	Scotland	Gavriel et al, 1998
	Biofilm, Water - WDS	USA	Chauret et al, 2001
	Biofilm - WDS	Australia	Bomo et al, 2004
	Water - WDS	Korea	Lee et al, 2006
	Biofilm, Water - WDS	South Africa	September et al, 2007
	Water - WDS	Brasil	Razzolini et al, 2008
	Biofilm - DWTP	Romania	Farkas et al, 2011
Vibrio spp. *V. cholerae*	Water - WDS	Korea	Lee et al, 2006
	Biofilm, Water - WDS	South Africa	September et al, 2004; 2007
	Biofilm - pond	Bangladesh	Alam et al, 2007
	Water - reservoirs	Sudan	Shanan et al, 2011
Mycobacterium spp.	Biofilm - WDS	Germany	Schmeisser et al, 2003
	Biofilm - WDS	South Africa	September et al, 2004
	Water - WDS	Greece	Kormas et al, 2010
	Water - WDS	USA	Marciano-Cabral et al, 2010
Shigella spp., *Salmonella* spp.	Biofilm - WDS	Germany	Schmeisser et al, 2003
	Water - WDS	Korea	Lee et al, 2006
Campylobacter spp.	Water - WDS	Finland	Hänninen et al, 2002
	Raw water	France	Gallay et al, 2006
Helicobacter pylori	Biofilm - WDS	England	Watson et al, 2004
	Biofilm - WDS	Portugal	Bragança et al, 2005
Legionella pneumophila	Biofilm - WDS	Germany	Emtiazi et al, 2004
	Water - WDS	The Netherlands	Diederen et al, 2007
	Biofilm - WDS	Portugal	Menaia et al, 2008
	Water - WDS	USA	Marciano-Cabral et al, 2010

Table 1. Pathogenic and opportunistic pathogenic bacteria detected in association with drinking water; WDS – water distribution systems, DWTP - drinking water treatment plant.

Experimental studies emphasized on bacteria ability of colonization, survival and multiplication in water associated biofilms, followed by dispersion in water phase in a planktonic state. The findings of Banning et al. (2003) suggested that the ability of *P. aeruginosa* to survive longer than *E. coli* in water associated biofilm could not be attributed to the association with the biofilm, rather than to the ability to utilize a wider range of organic molecules as carbon and energy sources compared to other *Enterobacteriaceae*. An increment in available nutrients may reduce *E. coli* survival in enhanced competition for nutrients and increased antagonism by the indigenous microbial population.

Lehtola et al. (2007) investigated the survival of faecal indices versus pathogenic bacteria and viruses, in drinking water biofilms experimentally infested with *E. coli*, *L. pneumophila*, *Mycobacterium avium* and canine calcivirus (as a surrogate for human norovirus). The results proved that pathogenic bacteria and virus particles entering water distribution systems can survive in biofilms for weeks, even in conditions of high-shear turbulent flow and may pose a risk to the consumers. Meanwhile, *E. coli* registered a limited survival to a few days in water and in biofilms, being a poor indicator of certain pathogens in biofilms. The study also showed that standard culture methods may seriously underestimate the real numbers of bacteria in water and biofilms.

Comparative evaluation of classical techniques involving bacterial growth on specific selective media and molecular methods based on 16s rDNA sequence identity reveals a high discrepancy between what was expected to grow and the species isolated from specific selective growth media. Bacterial analyses of water based on selective isolation and culturing approach is recommended to be interpreted with caution (September et al, 2007).

Experimental studies revealed also low detectable numbers by culture-based technique in case of potable water biofilms infected with *Campylobacter jejuni* (Lehtola et al, 2006). *C. jejuni* and *C. coli* waterborne epidemics registered in Finland (Hänninen et al, 2000) and France (Gallay et al, 2000) were associated to consumption of contaminated tap water with origin in polluted sources.

Severe outbreaks such as cholera caused by the ingestion of water contaminated with *Vibrio cholerae*, typhoid and paratyphoid enteric fevers caused by *Salmonella enterica* subsp. *enterica* serovar *Typhi*, respective serovar *Paratyphi*, shigellosis due to infections with *Shigella* species still occur in countries with insufficient access to safe water. But even in developed countries, outbreak events involving emerging pathogenic bacteria like *Legionella pneumophila*, waterborne E.coli O157:H7 and foodborne *E.coli* O104:H4 demonstrate the microbes' versatility and the fragility of humanity's victory over the nature.

2.1.2 Occurrence of protozoa in drinking water and associated biofilms

The food web in drinking water microbial consortia is based on heterotrophic bacteria, the next trophic level being represented by protozoa. Species of parasitic protozoa, including free living amoebae associated with infections in humans have been isolated from source waters and drinking water (table 2). Their presence represents a double threat to human health, being also related to amoeba-resisting bacteria, such as *Legionella* spp. and *Mycobacterium* spp., which proliferate in protozoa thus increasing the probability of causing diseases in humans (Marciano-Cabral et al, 2010).

Some protozoa, for example *Giardia* spp. and *Cryptosporidium* spp. may persist to hostile environment in drinking water, resist to different disinfection procedures and accumulate in biofilms under the form of cysts, respective oocysts. Experimental introducing *Cryptosporidium* oocysts for the prediction of behaviour in drinking water distribution system showed surface attachment and subsequent intermittent detachment, with exposure to high doses of chlorine (20mg/L) needed for the removal of substantial numbers of oocysts attached to pipe walls (Warneke et al, 2006). The study of Helmi et al. (2008) investigating the interaction of *Giardia lamblia* and *Cryptosporidium parvum* (oo)cysts in drinking water biofilms, revealed that protozoa are able to attach in biofilm matrix from the first day and survive extended periods of time, longer for *Cryptosporidium*. Viable (oo)cysts were recovered from biofilm and water phase for the whole period of investigation, of 34 days, turbulent shear stress influencing the detachment.

Protozoa	Samples type/Origin	Country	References
Flagellates: *Giardia lamblia*	Filtered water - DWTP Water - WDS Water - WDS Water - WDS	USA Canada Australia Spain	LeChevallier et al, 1991 Chung et al, 1998 Hellard et al, 2001 Carmena et al, 2007
Apicomplexa (Sporozoans): *Cryptosporidium parvum*	Filtered water - DWTP Water - WDS Water - WDS	USA Canada Spain	LeChevallier et al, 1991 Chung et al, 1998 Carmena et al, 2007
Amoebae: *Naegleria fowleri* *Acanthamoeba* spp. *Hartmannella* spp. *Vahlkampfia* spp.	 Well water Biofilm, Water - WDS Biofilm, Water - WDS Water reservoir	 USA USA USA Sudan	 Blair et al, 2008 Marciano-Cabral et al, 2010 Shoff et al, 2010 Shanan et al, 2011

Table 2. Protozoa detected in raw water sources, water treatment plants (DWTP) and drinking water networks (WDS) and associated biofilms.

2.1.3 Occurrence of viruses in drinking water and associated biofilms

Sources of drinking water were investigated for the presence of enteric viruses, especially when gastrointestinal outbreaks occurred, and the results revealed episodes of faecal contamination in raw water. Epidemiological studies conducted supported the association between drinking water consumption and illness (table 3).

Viruses	Samples type/Origin	Country	References
Hepatitis A virus Hepatitis E virus	Well water Water - WDS	USA India	Bloch et al, 1990 Hazam et al, 2010
Noroviruses	Well water Spring water Groundwater Groundwater	USA Finland New Zeeland Korea	Parshionikar et al, 2003 Maunula et al, 2005 Hewitt et all, 2007 Koh et al, 2011
Coxsakie A viruses	Raw water	Taiwan	Hsu et al, 2009
Adenoviruses Rotaviruses	Drinking water sources	West Africa	Verheyen et al, 2009

Table 3. Viruses identified in raw water sources and water distribution networks (WDS).

The presence of enteric viruses associated with inadequate water supplies, poor sanitation and hygiene is mostly affecting developing countries (Ashbolt et al, 2004). Episodes of gastroenteritis caused especially by noroviruses attributed to contaminated drinking water have been reported also in developed countries. Inefficient raw water treatment and secondary contamination of distribution systems with sewage are of high concern, enteric viruses being generally more resistant than enteric bacteria to widely used free chlorine, chlorine dioxide and monochloramine disinfectants (LeChevallier & Au, 2004). Although no complete investigations regarding faecal indicators presence were performed in the considered studies (especially for intestinal enterococci as index of viruses), coliforms and *E. coli* have been detected in water samples in many cases of gastroenteritis outbreaks investigated (Parshionikar et al, 2003; Hewitt et al, 2007; Koh et al, 2011).

There is no evidence of viruses ability of multiplication in environmental biofilms, but they may survive for extended periods of time trapped in the matrix, similarly to protozoan (oo)cysts, and be detached in water column, where remain inactive until they find a host. Experimental studies using pilot scale systems demonstrated the ability of viruses to attach and accumulate into drinking water biofilms within one hour after inoculation, while their detachment in water phase is influenced by flow velocity (Lehtola et al, 2007; Helmi et al, 2008). The viral genomes were detected in biofilms over the whole period of both experiments (for 21, respectively 34 days). Helmi and co-workers, investigating the poliovirus infectivity, recovered the infectious viruses only for 6 days, when flow velocity increment from laminar to turbulent regimen was applied, concluding that detection of viral genome in biofilms is not sufficient to assess a risk associated with the presence of infectious particles.

2.1.4 Occurrence of fungi in drinking water and associated biofilms

Initially considered to be airborne, fungal infections in immunocompromised patients hospitalized in controlled atmospheric conditions raised the hypothesis of waterborne origin of aspergillosis (Anaissie & Costa, 2001). Opportunistic pathogens, potentially causing superficial or systemic infections, allergenic or toxigenic species of fungi (yeasts and moulds) have been isolated from drinking water worldwide, their presence being primary attributed to the ability of surfaces colonization as biofilms (table 4).

Fungi	Samples type/Origin	Country	References
Paenicillium spp.	Biofilm - WDS	USA	Doggett, 2000
Aspergillus spp.	Water - WDS	Germany	Göttlich et al, 2002
Cladosporium spp.	Biofilm, water - WDS	USA	Kelley et al, 2003
Epicoccum spp.	Water - WDS	Norway	Hageskal et al, 2006
Alternaria spp.	Water - WDS	Portugal	Gonçalves et al, 2006
Trichoderma spp.	Water WDS	Brazil	Pires-Gonçalves et al, 2008
Acremonium spp.	Water - WDS	Australia	Sammon et al, 2010
Exophiala spp.			
Phialophora spp.			
Fusarium spp.			
Mucor spp.			
Candida spp.			

Table 4. Fungi identified in drinking water distribution systems (WDS) and associated biofilms.

In some studies, the correlation with standard hygiene indicators was not found (Göttlich, 2002), other authors described negative correlations between bacteria and filamentous fungi, which may be explained either by competition for nutrients either by inhibiting toxins produced (Gonçalves et al, 2006) while in other investigations positive significant correlations were found between the presence of filamentous fungi, yeasts and bacteria in drinking water (Sammon et al, 2010). Regarding filamentous fungi behaviour in water distribution systems, deposition is attributed to highly resistant spores, while mycotoxins, taste and odour changing compounds producing implies germination and hyphal growth in biofilms. The occurrence of fungi in drinking water systems may have significant impact due to health effects of mycotoxins (such as aflatoxins): mutagenic, teratogenic, oestrogenic, carcinogenic and allergenic, although no reports of disease attributed to mycotoxins produced in the water distribution systems have been reported (Sonigo et al, 2011).

2.1.5 Occurrence of algae in drinking water and associated biofilms

Algae are assumed not to be characteristic to water distribution system biofilms due to the absence of light (Wingender and Flemming, 2011), but algal biomass is a major component of biofilms in surface source waters, water treatment and storage, in areas exposed to air and light. Experimental research designed by Chrisostomou et al (2009) emphasized on air-dispersed phytoplankton diversity and colonization potential of algal taxa in drinking water reservoir systems. Algal communities are associated to biofilms and may support bacterial growth, for example *Legionella* species (Declerck, 2010). Few recent studies investigating the presence of algae in drinking water are available (table 5).

Algae	Samples type/Origin	Country	References
Oocystis spp. *Xenococcus* spp.	Water - WDS	Spain	Codony et al, 2003
Anabaena spp. *Microcystis* spp. *Oscillatoria* spp. and many more	Water - WDS	Argentina	Ricardo et al, 2006
Microcystis aeruginosa *Chroococcus dispersus*	Water reservoirs	Greece	Lymperopoulou et al, 2011

Table 5. Algae identified in drinking water distribution systems (WDS).

Algal toxins, of which the most dangerous for humans is cyanobacterial microcystin, are considered chemical hazards in drinking water, especially when open-air reservoirs are used in water storage (Lymperopoulou et al, 2011). Algal growth and eutrophication in surface waters are widely investigated, with respect to ecological effects. In drinking water sources and throughout the water treatment process, distribution and storage, algal blooms raise issues about toxins releasing and aesthetic problems inducing, such as colour and smell. Algal removal in drinking water treatment is recommended to be carefully performed, in order not to disrupt the cells and release toxins in drinking water (LeChevallier and Au, 2004). Epidemiological studies are conducted worldwide in order to demonstrate the evidence of algal toxins in the environment and to evaluate their relatedness to illness in humans. Possible linkages between algae toxins in drinking water and health effects, including liver problems and diarrhoea in children were indicated by a survey in Namibia, although microcystin never exceeded the tolerable daily intake (Gunnarsson and Sanseovic, 2001).

3. Drinking water and associated biofilms – Chemical aspects

Detrimental effects of biofouling in drinking water distribution systems include chemical aspects, involving organic and inorganic compounds produced by the microorganisms inhabiting water phase, biofilms and sediments. Different volatile compounds, organic and inorganic acids, metal oxides and enzymes resulted in microbial metabolism or decay may cause aesthetic problems in water: colour, taste and odours and may also have an impact on the substratum, leading to microbially influenced corrosion.

3.1 Drinking water aesthetic problems

Aesthetic and organoleptic characteristics of water may be affected by a series of chemical substances, resulting in colour, odour and taste degradation. Such substances originate in microbial activity and decomposition in source waters and in distribution systems, disinfectants used in water treatment, materials used in pipes and joints in water networks. A list of these substances, related to microbial activity and decay that may be produced in the journey of drinking water from drinking water sources to the tap, that may influence consumers perception, is presented in table 6 (after the UK Environment Agency, 2004). These chemical compounds are usually attributed to microbial biofilms associated to drinking water processing and distribution.

Investigating the sources of taste and odour in drinking water in order to find their sources and mitigation strategies, Peter (2008) concluded that low concentrations in chlorine residuals, stagnant water, plastic pipes and particles accumulation in distribution systems may increase the generation of taste and odour compounds by favouring biofilm formation and microbial activity. Other sources of aesthetic problems in water may reside in the activity of bacteria involved in sulphur cycle, producing sulphur odours and yellow discoloration (US EPA, 2002). Oxidation and reduction of soluble metals may produce metal oxides, leading to consumer complaints about the metallic taste and yellow, black or brown staining water (Cerrato et al, 2006).

3.2 Microorganisms – Surface interactions and microbially influenced corrosion

Biofouling proved to be interdependent on surface characteristics. Investigations of microbial reversible and irreversible attachment in primary or secondary colonization and in drinking water biofilms composition concluded as following:

- The hydrophobic/hydrophilic properties of the substrate are influencing biofilm formation. Exopolysacharides produced by some bacteria facilitate cell adhesion to hydrophilic surfaces, while exopolymers of other bacteria may show a preference for hydrophobic substrata (Beech et al, 2005).

Regarding the influence of the substratum on biofilm composition, copper materials appear to be colonized just by *L. pneumophila* in low numbers, inhibiting *P. aeruginosa* integration, while drinking water biofilms on elastomeric and polyethylene materials proved to be a better support for pseudomonads (Moritz et al, 2010).

- Pipe materials may be corroded, influencing disinfection effectiveness: corrosion products in iron pipes react with free chlorine and lead to residual disinfectants depletion. LeChevallier et al. (1987) detected high concentrations of coliforms only in tubercles

formed on iron pipes and suggested few possible explanations: coliform growth stimulated by iron oxides; nutrient syntropy; sourface roughness; protection from disinfection. Iron pipes may be a better support for fungi also, when compared to PVC pipes (Doggett, 2000).

Drinking water flowing through PVC pipes contains three times the aqueous concentration of soluble manganese and 35 times the concentration of total manganese than present in the drinking water transported by iron pipes (Cerrato et al, 2006).

Microorganisms	Chemical substances produced	Aesthetic effects
Microbial decomposition	Indole, skatole, putrescine, cadaverine, β-phenylethylamine, butyric, propionic and stearic acids	Fishy, grassy, woody tastes Faecal, rotten, cheese, pungent odours
Algae decomposition	Mercaptan, dimethyl sulphide, polysulphides	Fishy, swampy, septic odours
Algae decomposition/activity	n-hexanal, n-heptanal, isomers of decadienal sulphur compounds, terpenes, aromatic compounds, esters	Fishy odours Rotten eggs odours Aromatic odours
Pseudomonas spp. *Flavobacterium* spp. *Aeromonas* spp. *Paenicillium caeseicolum*	Dimethyl polysulphides	Swampy odours
Fungi *Chaetomium globosum* *Basidiobolus ranarumi* *Actinomycetes*	Geosmin Cadin-4-ene-1-ol 2-isopropyl-3-methoxypyrazine	Earthy, musty taste and odour Woody, earthy odour Musty, mouldy potato odour
Actinomycetes*:* *Streptomyces* spp. *Nocardia* spp. *Microbiospora* spp Cyanobacteria*:* *Anabaena* spp. *Microcystis* spp. *Oscillatoria* spp. *Aphanizomenon* spp. Algae*:* Chlorophyceae Bacillariophyceae	Geosmin 2-methylsorboneol	Earthy, musty taste and odours
Sulphur oxidizing/reducing bacteria Sulphate reducing bacteria	Sulphuric acid, sulphates, sulphur, methyl mercaptan, hydrogen sulphide Metal sulphides (ferrous sulphide)	Rotten eggs, rotten cabbage odours Yellow, brown, black staining
Metals oxidizing/ reducing bacteria	Metal oxides	Rusty or metallic taste Brown, black staining

Table 6. Chemical compounds produced by microbial decomposition and metabolism, affecting taste and odour of drinking water.

Microbially influenced corrosion represents another undesirable impact of biofilms associated to drinking water treatment and distribution, involving metallic or non-metallic materials deterioration as a result of pipes inner surface biofouling.

Physiological groups of bacteria classified on account of the ability to use different substrates in their nutrition or in respiration are summarized in table 7 (Drăgan-Bularda & Kiss, 1986; Drăgan-Bularda & Samuel, 2006; Muntean, 2009). Their representative species may belong to microbial communities of source waters and sediments, enter drinking water

treatment plants and distribution systems in a planktonic state and adhere to surfaces or become members of established biofilms (Costerton, 1994). Their metabolites have significant impacts on drinking water quality, either being released in bulk water where may react with other compounds, for example with disinfectants, leading to toxic disinfection-by products (as trihalomethanes), or by remaining in biofilm matrix where acting upon pipes surfaces and inducing corrosion.

Physiologycal groups of bacteria	**Representatives**	**Metabolites produced**
Ammonifying bacteria	*Bacillus* spp. *Clostridium* spp. *Pseudomonas* spp. *Burkholderia* spp.	Ammonium Ammonia
Nitrosifiers (Ammonia oxidizing bacteria)	*Nitrosomonas* spp. *Nitrocystis* spp. *Nitrospira* spp. *Nitrosolobus* spp. *Nitrosovibrio* spp.	Nitrite ions
Nitrifying bacteria (Nitrite oxidizing bacteria)	*Nitrobacter* spp *Nitrococcus* spp. *Nitrospira* spp. *Nitrospina* spp.	Nitrate ions
Denitrifying bacteria	*Paracoccus denitrificans* *Pseudomonas stutzeri* *Thiobacillus denitrificans* *Alcaligenes* spp. *Bacillus* spp.	Nitrous oxide Nitrogen
Sulphur reducing bacteria	*Desulfuromonas* spp. *Proteus* spp.	Hydrogen sulphide
Sulphate reducing bacteria	*Desulfovibrio desulfuricans* *Desulfovibrio sulfodismutans* *Desulfotomaculum* spp. *Desulfonema* spp. *Desulfosarcina* spp. *Desulfobacter* spp. *Desulfococcus* spp. *Desulfomicrobium* spp.	Hydrogen sulphide
Sulphur oxidizing bacteria:	*Thiobacillus* spp. *Sulfolobus* spp. *Beggiatoa* spp. *Thiothrix* spp.	Sulphuric acid Sulphates Sulphur
Iron reducing bacteria	*Sphaerotilus natans* *Leptothrix ochracea* *Crenothrix polyspora*	Iron (Fe^{2+}) oxides
Iron oxidizing bacteria	*Galionella feruginea* *Ferrobacillus ferooxidans* *Thiobacillus ferooxidans*	Iron (Fe^{3+}) oxides
Manganese oxidizing/reducing bacteria	*Sphaerotilus discophorus* *Pseudomonas* spp. *Metallogenium* spp. *Pedomicrobium* spp. *Bacillus* spp. *Micrococcus* spp. *Vibrio* spp.	Manganese oxides

Table 7. Physiological groups of bacteria, their representatives and metabolites.

Chemical and enzymatic microbial products resulted in biofilms activity may induce corrosion and related effects by different mechanisms:

- oxygen concentration cells and anaerobic sites generation (promoting growth of anaerobic bacteria);
- formation of iron concentration cells by the activity of iron and manganese oxidizing bacteria;
- metabolites such as acids produced by bacteria have corrosive action upon the surface;
- production of depolarizing enzymes within the biofilm matrix, which may persist longer than viable cells;
- exopolymers produced by slime forming bacteria stimulate biofilm formation and biomass accumulation;
- the binding capacity of biofilm matrix which may lead to deposits accumulation with clogging effects (Beech et al, 2005; Coetser and Cloete, 2005).

Some of the recommended strategies in drinking water associated biofilm control are: source waters protection, appropriate treatment, infrastructure contamination prevention, pipes and reservoirs maintenance, corrosion control, appropriate disinfection practices, nutrient levels reducing, water quality monitoring, personnel training, water safety plans implementation.

We are still living in an age of surfaces, even the remark was first said by Oscar Wild's character in 1895. Having in mind the virtual idea of self-cleaning surfaces, researchers in nanotechnology field are targeting innovative repellent materials with a wide range of applications, for the biofouling control. The superhydrophobicity models such as "the lotus effect" characterizing the lotus (*Nelumbo nucifera*) leaf, offered by natural patterns are investigated at nanoscale. The interdependence between surface roughness, reduced particle adhesion and water repellence proved to be the keystone in the self-cleaning mechanism of many biological surfaces (Barthlott & Neinhuis, 1997).

4. Conclusions

The present review emphasize on the following recent and relevant findings:

- Biofilms associated with drinking water are ubiquitous, harbouring bacterial pathogens, opportunistic pathogens, parasitic protozoa, viruses, toxins releasing fungi and algae;
- Microbial consortia in contact with drinking water have significant impacts upon water quality and may threat human health when contamination events occur;
- Access to safe water continues to be a target for developing countries, unfulfilled at the moment;
- Even in developed countries, where substantial efforts are submitted in order to ensure population's access to a high quality drinking water, microbial versatility represents an endless source of problems, with respect to opportunistic pathogens emergence;
- Microbial communities in water networks and biofilms represent complex ecosystems; their ecology is influenced by a series of abiotic and biotic factors: raw water sources quality, temperature, flow rate and system hydraulics, nutrient concentration, pipe material, particles accumulation, ingress and intrusion, water treatment, water disinfection and microbial interactions;
- Further research is needed in order to understand attached microbial consortia for biofouling prevention and control in drinking water industry, as a matter of public security.

5. References

Alam, M.; Sultana, M.; Nair, G.B.; Siddique, A.K.; Hasan, N.A.; Sack, R.B.; Sack, D.A.; Ahmed, K.U.; Sadique, A.; Watanabe, H.; Grim, C.J.; Huq, A. & Colwell, R.R. (2007). Viable but nonculturable *Vibrio cholerae* O1 in biofilms in the aquatic environment and their role in cholera transmission. *Proceeding of the National Academy of Sciences of the USA*, Vol.4, No.5, pp. 17801-17806, www.pnas.org/cgi/content/full/0705599104/DC1

Allen, M.J. (2011). *Escherichia coli* – the most relevant microbial public health indicator for drinking water. *Faecal indicators: problem or solution?* Conference presentation, Edinburgh, UK

Anaissie, E.J. & Costa, S.F. (2001). Nosocomial aspergillosis is waterborne. *Clin Infect Dis*, Vol.33, pp. 1546-1548

Ashbolt, N.J. (2004). Microbial contamination of drinking water and disease outcomes in developing countries. *Toxicology*, Vol.198, pp. 229-238

Barthlott, W. & Neinhius, C. (1997). Purity of the sacred lotus, or escape from contamination in biological surfaces. *Planta*, Vol.202, pp. 1-8

Banning, N.; Toze, S. & Mee, B.J. Persistence of biofilm-associated *Escherichia coli* and *Pseudomonas aeruginosa* in groundwater and treated effluent in a laboratory model system. *Microbiology*, Vol.149, pp. 47-55

Beech, I.B.; Sunner, J.A. & Hiraoka, K. (2005). Microbe-surface interactions in biofouling and biocorrosion processes. *Int Microbiol*, Vol.8, pp. 157-168

Blair, B; Sarkar, P.; Bright, K.R.; Marciano-Cabral, F. & Gerba,C.P. (2008). *Naegleria fowleri* in well water. *Emerg Infect Dis*, Vol.14, No.9, pp. 1499-1500

Bloch, A.B.; Stramer, S.L.; Smith, J.D.; Margolis, H.S.; Fields, H.A.; McKinley, T.W.; Gerba, C.P.; Maynard, J.E. & Sikes, R.K. (1990). Recovery of hepatitis A virus from a water supply responsible for a common source outbreak of hepatitis A. *Am J Public Health*, Vol.80, No.4, pp. 428-430

Bomo, A.M.; Storey, M.V. & Ashbolt, N.J. (2004). Detection, integration and persistence of aeromonads in water distribution pipe biofilm. *J Water Health*, Vol.2, No.2, pp. 83-96

Bragança, S.M.; Azevedo, N.F.; Simões, L.C.; Vieira, M.J. & Keevil, C.W. (2005). Detection of *H. pylori* in biofilms formed in a real drinking water distribution system using peptide nucleic acid fluorescence in *situ* hybridization. *Biofilm Club*, pp. 231-239

Carmena, D.; Aguinagalde, X.; Ziggoraga, C.; Fernández-Crespo, J.C. & Ocio, J.A. (2007). Presence of *Giardia* cysts and *Cryptosporidium* oocysts in drinking water supplies in northern Spain. *J Appl Microbiol*, Vol.102, pp. 619-629

Cerrato, J.M.; Reyes, L.P.; Alvarado, C.N. & Dietrich, A.M. (2006). Effect of PVC and iron materials on Mn(II) deposition in drinking water distribution systems. *Water Res*, Vol.40, pp. 2720-2726

Chambless, J.D.; Hunt, S.M. & Stewart, P.S. (2005). A three-dimensional computer model of four hypothetical mechanisms protecting biofilms from antimicrobials. *Appl Environ Microbiol*, Vol.72, No.3, pp. 2005-2013

Characklis, W.G. (1988). *Bacterial regrowth in distribution systems*, Project Report, American Water Works Association, USA, http://waterrf.org/ProjectsReports/ExecutiveSummaryLibrary/90532_102_profiles.pdf

Chauret, C.; Volk, C.; Creason, R.; Jarosh, J.; Robinson, J. & Wrnes, C. (2001). Detection of *Aeromonas hydrophila* in a drinking-water distribution system: a field and pilot study. *Can J Microbiol,* Vol.47, No.8, pp. 782-786

Chrisostomou, A.; Moustaka-Gouni, M.; Sgardelis, S. & Lanaras, T. (2009). Air-dispersed phytoplankton in a Mediterranean river-reservoir system (Aliakmon-Polyphytos, Greece). *J Plankton Res*, Vol.31, pp. 877-884

Chung, E.; Aldom, J.E.; Chagla, A.H.; Kostrzynska, M.; Palmateer, G.; Trevors, J.T.; Unger, S. & De Grandis, S. (1998). Detection of *Cryptosporidium parvum* oocysts in municipal water samples by the polymerase chain reaction. *J Microbiol Meth*, Vol.33, No.2, pp. 171-180

Codony, F.; Miranda, A.M. & Mas, J. (2003). Persistence and proliferation of some unicellular algae in drinking water systems as a result of their heterotrophic metabolism. *Water SA*, Vol.29, No.1, pp. 113-116, http://www.wrc.org.za

Coetser, S.E. & Cloete, T.E. (2005). Biofouling and biocorrosion in industrial water systems. *Crit Rev Microbiol*, Vol.31, No.4, pp. 213-232

Costerton, J.W.; Cheng, K.J.; Gessey, G.G.; Ladd, T.I.; Nickel, J.C.; Dasgupta, M. & Marrie, T.J. (1987). Bacterial biofilms in nature and disease. *Annu Rev Microbiol* , Vol.41, pp. 435-464

Costerton, J.W. (1994). Structure of biofilms, In: *Biofouling and biocorrosion in industrial water systems*, Geesey, G.G.; Lewandowski, Z. & Flemming, H.C. (Ed.) CRC Press, ISBN 0 87371 928 X, USA

Declerck, P. (2010). Biofilms: the environmental playground of *Legionella pneumophila*. *Environ Microbiol*, Vol.12, No.3, pp. 557-566

Diederen, B.M.W.; de Jong, C.M.A. & Aarts, I. (2007). Molecular evidence for the ubiquitous presence of *Legionella* species in Dutch tap water installations. *J Water Health*, Vol.5, No.3, pp. 375-383

Doggett, M.S. (2000). Characterization of fungal biofilms within a municipal water distribution system. *Appl Environ Microb*, Vol. 66, No. 3, pp. 1249-1251

Drăgan-Bularda, M. & Kiss, S. (1986). *Soil microbiology* (in Romanian), Babeş-Bolyai University of Cluj-Napoca Press, Romania

Drăgan-Bularda, M. & Samuel, A.D. (2006). *General microbiology* (in Romanian), University of Oradea Press, ISBN 10 973 759 056 2

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 Res*, Vol.38, pp. 1197-1206

Exner, M.; Vacata, V. & Gebel, J. (2003). Public health aspects of the role of HPC – an introduction, In: *Heterotrophic plate counts and drinking water safety. The significance of HPCs for water quality and human health,* Bartram, J.; Cotruvo, J.; Exner, M.; Fricker, C. & Glasmacher, A., (Ed.), IWA Publishing, ISBN 1 84339 025 6, London, UK, pp. 12-19

Farkas, A.; Drăgan-Bularda, M.; Ciatarâş, D.; Bocoş, B. & Ţigan, Ş. (2011): Opportunistic pathogens and faecal indicators assessment in drinking water associated biofilms in Cluj, Romania (in preparation)

Fields, B.S.; Benson, R.F. & Besser, R.E. (2002). *Legionella* and Legionnaire's disease: 25 years of investigation. *Clin Microbiol Rev*, Vol.15, No.3, pp. 506-526

Flemming, H.C.; Percival, S.L. & Walker, J.T. (2002). Contamination potential of biofilms in water distribution systems. *Water Supp*, Vol.2, No.1, pp. 271-280

Flemming, H.C. (2008). Why microorganisms live in biofilms and the problem of biofouling. *Biofouling*, pp. 3-12, doi: 10.1007/7142

Gallay, A.; De Valk, H.; Cournot, M.; Ladeuil, B.; Hemery, C.; Castor, C.; Bon, F.; Mégraud, F.; Le Cann, P. & Desenclos, J.C. (2006). A large multi-pathogen waterborne community outbreak linked to faecal contamination of a groundwater system, France, 2000. *Clin Microbiol Infec*, Vol.12, No.6, pp 561-570

Gavriel, A.A.; Landre, J.P.B. & Lamb, A.J. (1998). Incidence of mesophilic *Aeromonas* within a public drinking water supply in north-east Scotland. *J Appl Microbiol*, Vol.84, pp. 383-392

Gillings, M.R.; Xuejun, D.; Hardwick, S.A. & Holley, M.P. (2009). Gene cassettes encoding resistance to quaternary ammonium compounds: a role in the origin of clinical class 1 integrons? *The ISME Journal*, Vol. 3, pp. 209-215

Glasmacher, A.; Engelhart, S. & Exner, M. (2003). Infections from HPC organisms in drinking-water amongst the immunocompromised, In: *Heterotrophic Plate Counts and Drinking-water Safety*, Bartram, J.; Cotruvo, J.; Exner, M.; Fricker, C. & Glasmacher, A., (Ed.), IWA Publishing, ISBN 1 84339 025 6, London, UK, pp. 137-145

Gonçalves, A.B.; Paterson, R.M. & Lima, N. (2006). Survey and significance of filamentous fungi from tap water. *Int J Hyg Environ Health*, Vol.209, pp. 257-264

Göttlich, E.; van der Lubbe, W.; Lange, B.; Fiedler, S.; Melchert, I.; Reifenrath, M.; Flemming, H.C. & Hoog, S. (2002). Fungal flora in groundwater-derived public drinking water. *Int J Hyg Environ Health*, Vol.205, No.4, pp. 269-279

Gunnarsson, H. & Sanseovic, A.M. (2001). *Possible linkages between algae toxins in drinking water and related illnesses in Windhoek, Namibia*, Thesis, Kristianstad University, Sweden, hkr.divaportal.org/smash/get/diva2:231226/FULLTEXT01

Hageskal, G.; Knutsen, A.K.; Gaustad, P.; de Hoog, G.S. & Skaar, I. (2006). Diversity and significance of mold species in Norvegian drinking water. *Appl Environ Microbiol*, Vol.72, No.12, pp. 7586-7593

Hazam, R.K.; Singla, R.; Kishore, J.; Singh, S.; Gupta, R.K. & Kar, P. (2010). Surveillance of hepatitis E virus in sewage and drinking water in a resettlement colony of Delhi: what has been the experience? *Arch Virol*, Vol.155, No.8, pp. 1227-1233

Hänninen, M.L.; Haajanen, H.; Pummi, T.; Wermundsen, K.; Katila, M.L.; Sarkkinen, H.; Miettinen, I. & Rautelin, H (2003). Detection and typing of *Campylobacter jejuni* and *Campylobacter coli* and analysis of indicator organisms in three waterborne outbreaks in Finland. *Appl Environ Microb*, Vol.69, No.3, pp. 1391-1396

Hellard, M.E.; Sinclair, M.I.; Forbes, A.B. & Fairley, C.K. (2001). A randomized, blinded, controlled trial investigating the gastrointestinal health effects of drinking water quality. *Environ Health Persp*, Vol.109, No.8, pp. 773-778

Helmi, K.; Skraber, S.; Gantzer, C.; Williame, R.; Hoffmnn, L. & Cauchie, H.M. (2008). Interactions of *Cryptosporidium parvum*, *Giardia lamblia*, vaccinal poliovirus type 1 and bacteriophages φX174 and MS2 with a drinking water biofilm and a wastewater biofilm. *Appl Environ Microb*, Vol.74, No.7, pp. 2079-2088

Hewitt, J.; Bell, D.; Simmons, G.C.; Rivera-Aban, M.; Wolf, S. & Greening, G.E. (2007). Gastroenteritis outbreak caused by waterborne Norovirus at a New Zeeland ski resort. *Appl Environ Microb*, Vol.73, No.24, pp. 7853-7857

Hsu, B.M.; Chen, C.H.; Wan, M.T.; Chang, P.J & Fan, C.W. (2009). Detection and identification of enteroviruses from various drinking water sources in Taiwan. *J Hydrol*, Vol.365, No.1-2, pp. 134-139

Hunter, P.R.; Payment, P.; Ashbolt, N & Bartram, J. (2003). Assessment of risk, In: *Assessing microbial safety of drinking water*, IWA Publishing, ISBN 1 84339 036 1, London, UK

Juhna, T.; Birzniece, D.; Larsson, S.; Zulenkovs, D.; Sharipo, A.; Azevedo, N.F.; Menard-Szczebara, F.; Castagnet, S.; Feliers, C. & Keevil, C.W. (2007). Detection of *Escherichia coli* in biofilms from pipe samples and coupons in drinking water distribution networks. *Appl Environ Microb* Vol.73, No.22, pp. 7456–7464

Kelley, J.; Kinsey, G.; Peterson, R. & Brayford, D. (2003). Identification and control of fungi in distribution systems. *Water Quality Technology Conference Proceedings*, Awwa Research Foundation Denver, USA, www.biosan.com/pubs/Identification Significance.pdf

Koh, S.J.; Cho, H.G.; Kim, B.H. & Choi, B.Y. (2011). An outbreak of gastroenteritis caused by Norovirus-contaminated groundwater at a waterpark in Korea. *Infectious Diseases, Microbiology & Parasitology*, Nr.26, pp. 28-32

Kormas, K.A.; Neofitou, C.; Pachiadaki, M. & Koufostathi, E. (2010). Changes of the bacterial assemblages throughout an urban drinking water distribution system. *Environ Monit Assess*, No.165, pp. 27-38

Latimer, J.; McLeod, C.; Jackson, R.; Bunch, J.; Graham, A.; Stokes S. & Poole, R. (2010). *Escherichia coli* biofilms: gene expression and elemental heterogeneity. *Biofilms 4 Conference Handbook*, Winchester, UK, pp. 86

LeChevallier, M.W.; Babcock, T.M. & Lee, R.G. (1987). Examination and characterization of distribution system biofilms. *Appl Environ Microb*, Vol.53, No.12, pp. 2714-2724

LeChevallier, M.W.; Norton, W.D. & Lee, R.G. (1991). *Giardia* and *Cryptosporidium* in filtered drinking water supplies. *Appl Environ Microb*, Vol.57, No.9, pp. 2617-2621

LeChevallier, M.W. & Au, K.K. (2004). *Water treatment and pathogen control*, IWA Publishing, ISBN 1 84339069 8, London, UK

Lee, D.G.; Kim, S.J. & Park, S.J. (2006). Effect of reservoirs on microbiological water qualities in a drinking water distribution system. *J Microbiol Biotechn*, Vol.16, pp. 1060-1067

Lehtola, M.J.; Pitkänen, T.; Miebach, L. & Miettinen, I.T. (2006). Survival of *Campylobacter jejuni* in potable water biofilms: a comparative study with different detection methods. *Water Sci Technol,* Vol.54, No.3, pp. 57-61

Lehtola, M.J.; Torvinen,E.; Kusnetsov, J.; Pitkänen, T.; Maunula,L.; von Bonsdorff, C.H.; Martikainen, P.J.; Wilks, S.A.; Keevil, C.W & Miettinen, I.T. (2007). Survival of *Mycobacterium avium*, *Legionella pneumophila*, *Escherichia coli*, and calciviruses in drinking water-associated biofilms grown under high-shear turbulent flow. *Appl Environ Microb*, Vol.73, No.9, pp. 2854-2859

Lymperopoulou, D.S.; Kormas, K.A.; Moustaka-Gouni, M. & Karagouni, A.D. (2011). Diversity of cyanobacterial phylotypes in a Mediterranean drinking water reservoir (Marathonas, Greece). *Environ Monit Assess*, Vol.173, pp.155-165

Marciano-Cabral, F.; Jamerson, M. & Kaneshiro, E.S. (2010). Free-living amoebae, *Legionella* and *Mycobacterium* in tap water supplied by a municipal drinking water utility in the USA. *J Water Health*, Vol.8, No.1, pp. 71-82

Martiny, A.C.; Jørgensen, 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. *Appl Environ Microb*, Vol.69, No.11, pp. 6899-6907

Maunula, L.; Miettinen, I.T. & von Bonsdorff, C.H. (2005). Norovirus outbreaks from drinking water. *Emerg Infect Dis*, Vol.11, No.11, pp. 1716-1721

Menaia, J.; Benoliel, M.; Lopes, A.; Neto, C.; Ferreira, E.; Mesquita, E.& Paiva, J. (2008). Assessment of Lisbon drinking water distribution network biofilm colonization and associated hazards. *Wa Sci Technol*, Vol.8, No.4, pp. 421-426

Moritz, M.M.; Flemming, H.C. & Wingender, J. (2010). Integration of *Pseudomonas aeruginosa* and *Legionella pneumophila* in drinking water biofilms grown on domestic plumbing materials. *Int J Hyg Environ Health*, Vol.213, No.3, pp. 190-197

Muntean, V. (2009). *General microbiology* (in Romanian), Babeş-Bolyai University of Cluj-Napoca Press, Romania

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

Parshionikar, S.U.; William-True, S.; Fout, G.S.; Robbins, D.E.; Seys, S.A.; Cassady, J.D. & Harris, R. (2003). Waterborne outbreak of gastroenteritis associated with a Norovirus. *Appl Environ Microb*. Vol. 69, No.9, pp. 5263-5268

Payment, P. & Robertson, W. (2004). The microbiology of piped distribution systems and public health, In: *Safe piped water: Managing Microbial Water Quality in Piped Distribution Systems*, Ainsworth, R. (Ed.), IWA Publishing, ISBN 1 84339 039 6, London, UK

Payment, P. & Locas, A. (2011). Pathogens in water: value and limits of correlation with microbial indicators. *Ground Water*, Vol.49, No.1, pp 4-11

Peter, A. (2008). *Taste and odor in drinking water: Sources and mitigation strategies*, PhD Thesis, Swiss Federal Institute of Technology Zurich, Switzerland, http://e-collection.ethbib.ethz.ch/eserv/eth:30628/eth-30628-02.pdf

Pires-Gonçalves, R.H.; Sartori, F.G.; Montanari, L.B.; Zaia, J.E.; Melhem, M.S.C.; Mendes-Giannini, M.J.S. & Martins C.H.G. (2008). Occurrence of fungi in water used at a haemodialysis centre. *Lett Appl Microbiol*, Vol.46, pp. 542-547

Razzolini, M.T.P.; Bari, M.; Sanchez, P.S. & Sato, M.I.Z. (2008). *Aeromonas* detection and their toxins in drinking water from reservoirs and drinking fountains. *J Water Health*, Vol.6, No.1, pp. 117-123

Reynolds, K.A.; Mena, K.D. & Gerba, C.P. (2008). Risk of waterborne illness via drinking water in the United States. *Rev Environ Contamin T*, Vol.192, pp. 117-158

Ricardo, E.; Leda, G. & Luis, F. (2006). Drinking water: problems related to water supply in Bahia Blanca, Argentina. *Acta Toxicol Argent*, Vol.14, No.2, pp. 23-30.

Riley, M.R.; Gerba, C.P. & Elimelech, M. (2011). Biological approaches for addressing the grand challenge of providing access to clean drinking water. *J Biol Eng*, Vol.5, No.2

Rusin, P.A.; Rose, J.B.; Haas, C.N. & Gerba, C.P. (1997). Risk assessment of opportunistic bacterial pathogens in drinking water. *Rev Environ Contam T*, Vol.152, pp. 57-83

Sammon, N.B.; Harrower, K.M.; Fabbro, L.D. & Reed, R.H. (2010). Incidence and distribution of microfungi in a treated municipal water supply system in sub-tropical Australia. *Int J Environ Res Public Health*, Vol.7, pp. 1597-1611

Schmeisser, C.; Stockigt, C.; Raasch, C.; Wingender, J.; Timmis, K.N.; Wenderoth, D.F.; Flemming, H.C.; Liesegang, H.; Schmitz, A.; Jaeger, K.E. & Streit, W.R. (2003). Metagenome survey of biofilms in drinking water networks. *Appl Environ Microb*, Vol.69, No.12, pp. 7298-7309

September, S.M.; Els, F.A.; Venter, S.N. & Brozel, V.S. (2007). Prevalence of bacterial pathogens in biofilms of drinking water distribution systems. *J Water Health*, Vol.5, No.2, pp. 219-227

Servais, P.; Anzil, A.; Gatel, D. & Cavard, J. (2004). Biofilm in the Parisian suburbs drinking water distribution system. *J Water Supply Res T*, Vol.53, pp. 313-324

Shanan, S.; Abd, H.; Hedenström, I.; Saeed, A. & Sandström, G. (2011). Detection of *Vibrio cholerae* and *Acanthamoeba* species from same natural water samples collected from different cholera endemic areas in Sudan. *BioMed Central Research Notes*, Vol.4, No.109, http://www.biomedcentral.com/1756-0500/4/109

Shoff, M.E.; Rogerson, A.; Kessler, K.; Schatz, S. & Seal, D.V. (2008). Prevalence of *Acanthamoeba* and other naked amoebae in South Florida domestic water. *J Water Health*, Vol.6, No.1, pp. 99-104

Sonigo, P.; De Toni, A. & Reilly, K. (2011). *A review of fungi in drinking water and the implications for human health*, Report, Bio Intelligent Service, France

Srinivasan, S. (2008). *Managing bacterial regrowth and presence in drinking water distribution systems*, Ph.D. thesis, University of Wisconsin-Madison, USA

Szewzik, U.; Szewzyk, R.; Manz, W. & Schleifer, K.H. (2000). Microbiological safety of drinking water. *Annu Rev Microbiol*, Vol.54, pp. 81-127

UK Environment Agency (2004). *The microbiology of drinking water (2004) - Part 11 – Taste, odour and related aesthetic problems*, London, UK, http://www.environment-agency.gov.uk/static/documents/Research/mdwpart112004_859972.pdf

US Environmental Protection Agency (1992). *Control of biofilm growth in drinking water distribution systems*, Seminar publication, Washington, USA, www.epa.gov/nrmrl/pubs/625r92001/625r92001.html

van der Kooij, D. (2003). Managing regrowth in drinking water distribution systems, In: *Heterotrophic plate counts and drinking water safety. The significance of HPCs for water quality and human health,* Bartram, J.; Cotruvo, J.; Exner, M.; Fricker, C. & Glasmacher, A., (Ed.), IWA Publishing, ISBN 1 84339 025 6, London, UK, pp. 199-232

Verheyen, J.; Timmen-Wego, M.; Laudien, R.; Boussaad, I.; Sen, S.; Koc, A.; Uesbeck, A.; Mazou, F. & Pfister, H. (2009). Detection of adenoviruses and rotaviruses in drinking water sources used in rural areas of Benin, West Africa. *Appl Environ Microb*, Vol.75, No.9, pp. 2798-2810

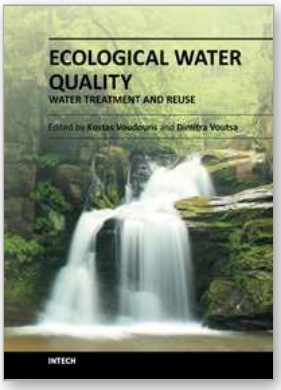
Warneke, M. (2006). *Cryptosporidium oocyst interactions with drinking water pipe biofilm*, Research report, Cooperative Research Center for Water Quality and Treatment, Adelaide, Australia, www.wqra.com.au/publications/document-search/ ?download=30

Watkins, J. & Jian, X. (1997). Cultural methods of detection for microorganisms: recent advances and successes. In: *The microbiological quality of water*, Sutcliffe, D.W (Ed.), Freshwater Biological Association, ISBN 0 900386 57 6, Ambleside, UK, pp 19-27

Watson, C.L.; Owen, R.J.; Said, B.; Lai, S.; Lee, J.V.; Surman-Lee, S. & Nichols, G. (2004). Detection of *Helicobacter pylori* but not culture in water and biofilm samples from drinking water distribution systems in England. *J Appl Microb*, No.97, pp. 690-698

White, D.C.; Kirkegaard, R.D.; Palmer, R.J.; Flemming, C.A.; Chen, G.; Leung, K.T.; Phiefer, C.B. & Arrage, A.A., (1999). The biofilm ecology of microbial biofouling, biocide resistance and corrosion, In: *Biofilms in the aquatic environment*, Keevil, C.W.; Godfree, A.; Holt, D. & Dow, C., (Ed.), The Royal Society of Chemistry, ISBN 0 85404 758 1, Cambridge, UK, pp. 120-130

Wingender, J. & Flemming, H.C. (2011). Biofilms in drinking water and their role as reservoir for pathogens. *Int J Hyg Environ Heal*, Vol.213, pp. 190-197



Ecological Water Quality - Water Treatment and Reuse
Edited by Dr. Voudouris

ISBN 978-953-51-0508-4
Hard cover, 496 pages
Publisher InTech
Published online 16, May, 2012
Published in print edition May, 2012

This book attempts to cover various issues of water quality in the fields of Hydroecology and Hydrobiology and present various Water Treatment Technologies. Sustainable choices of water use that prevent water quality problems aiming at the protection of available water resources and the enhancement of the aquatic ecosystems should be our main target.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Anca Farkas, Dorin Ciataras and Brandussa Bocos (2012). Biofilms Impact on Drinking Water Quality, Ecological Water Quality - Water Treatment and Reuse, Dr. Voudouris (Ed.), ISBN: 978-953-51-0508-4, InTech, Available from: http://www.intechopen.com/books/ecological-water-quality-water-treatment-and-reuse/biofilms-impact-on-drinking-water-quality

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821