The hidden life in drinking water installations: biofilms and viable-but-nonculturable bacteria of hygienic relevance

Biofilms in drinking water systems

Drinking water is not sterile and does not have to be so. Up to 10⁵ cells per mL can be present without any hygienic relevance. These cells have a tendency to attach to surfaces and form biofilms. Therefore, practically all surfaces in contact with water serve as a substratum for microbial biofilms, which cover these surfaces usually in a patchy form. And usually they do not represent any health risk for humans.

However, they can harbor microorganisms of hygienic relevance. This can occur occasionally and transiently and they may be guests in existing drinking water biofilms. Among these, *Legionella pneumophila*, *Pseudomonas aeruginosa* and others are reported (Flemming et al., 2013). Under favorable conditions, such bacteria can persist and even multiply in biofilms and, as a consequence, contaminate the drinking water (Wingender, 2011). From a health perspective, one should ensure that biofilms are not the source of hygienically-relevant bacteria in drinking water distribution and installation systems and that they do not give rise to concerns on human health.

In biofilms, the microorganisms can tolerate much higher concentrations of disinfectants than they can in suspended form in water (Davies, 2003). This property can lead to persistent problems in decontamination and sanitation efforts, last for years and cause considerable costs (Schauer et al., 2013). These include not only the direct costs for repeated disinfection measures and verification of success, but also working time, as well as measures for compensating situations of malfunction of the system. For example, in hospitals and retirement homes, point-of-use filters are employed at taps and shower heads; this is a quite expensive make-shift solution, and does not address the cause of the problem.

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The weakest link in the drinking water supply chain is the drinking water installation. This is the most complex and least controlled component in the chain. Pipe diameters can be very small, offering large surfaces in contact with water which can be colonized by biofilms; a great variety of plumbing materials is employed, not all of which
may comply with regulations; and the consumption patterns are irregular, including long stagnation periods (Flemming et al., 2013).

In order to avoid biofilm problems and to provide safe drinking water, the concept of “Biofilm Management” was developed. The aim is to take all means which are suited to reducing the risks which can arise from biofilms in drinking water installations. Variables which can be controlled are water quality, plumbing materials and operation conditions. Manipulation of these variables is possible by process-engineering, physical or chemical measures which can limit biofilm growth. Furthermore, the variables can be influenced by professional planning and operation of the system. Particular attention should be placed on avoiding no- and low-flow areas and dead legs. Fundamentally important for effective biofilm management is surveillance by significant sampling and analysis, in order to detect any risks on time and to assess the efficacy of measures and strategies in biofilm management. The major principles have been addressed in a guideline (VDI/DGVW 6023). Key features of such management are:

i. biologically stable drinking water,
ii. limitation of organic nutrients, with particular attention to those elastomeric plumbing materials which can leach out biodegradable substances such as plasticizers, anti-oxidants, anti-statics, paraffin and others
iii. close compliance of the state-of-the-art of operating drinking water installations, particularly the measures after stagnation periods
iv. representative surveillance.

Cases of persistent microbial contaminations and long-term problems of sanitation are known. In such situations, it has to be taken into account that the microorganisms can enter a viable-but-nonculturable state ("VBNC"," Oliver, 2005, 2010; Li et al., 2014). In this state, the organisms are not dead but cannot be detected by cultivation methods. They have the potential to resuscitate; therefore, they deserve special attention.

The viable-but-nonculturable state (VBNC)

The gold standard world-wide for determination of living bacteria in drinking water are the cultivation-dependent methods. They are based on the ability of microorganisms to multiply, grow in liquid media and form colonies on agar media. The opposing argument concludes that bacteria which no longer grow in or on nutrient media are dead, or at least irreversibly inactivated. Cultivation methods are of central relevance in practice – they are employed to assess the hygienic quality of drinking water, food and beverages as well as in the entire medical context. Their employment is extremely successful; among others they are the key for prevention of waterborne infectious diseases. However, these methods have their limits. It has been known for a long time that bacteria which do not grow are not necessarily dead. In this way, they can escape the “radar of surveillance” and enter a nonculturable state. In a specialized conference “How dead is dead?” in Bochum, 2009, this state was defined in the following way, based on the work of Oliver (2005, 2010):

“A bacterial cell in the VBNC state may be defined as one which fails to grow at the routine bacteriological conditions under which it would normally grow, but which is in fact alive and still has metabolic activity.”

This is a purely operational definition because it is based on the response of the cell to cultivation conditions under which it normally can be detected. The reason is an important phenomenon: in the VBNC state, microbial cells show practically no further growth metabolism. Therefore, they do not multiply and form colonies or cause turbidity in liquid media. But they can still keep their maintenance metabolism, which may include processes such as the replacement of cell components - membrane, cell wall etc., or the repairing of DNA damage due to UV irradiation or to the action of antimicrobials.

Entering the VBNC state can be understood as a survival mechanism. It may be a response to stress which might be detrimental to the cells if they continue to grow (Li et al., 2014). Such stress can be generated for example by disinfectants, toxic metal ions, nutrient depletion or unfavorable temperatures. Cultivation methods can then lead to false negative results because the cells are not dead but only inactive. This is already well known for Legionella pneumophila (Steinert et al., 1999; Alleron et al., 2008). For Pseudomonas aeruginosa, a facultative pathogen with increasing relevance for drinking water hygiene, less is known. However, first investigations indicate clearly that one can expect the same from that organism (Moritz et al., 2010; Flemming et al., 2013).

The problem with the VBNC state is that it can be transient and reversible. For L. pneumophila, it could be shown that the organism can return to the cultivable and also to the infectious state (Steinert et al., 1999), and recently, the same could be shown for P aeruginosa (Dwidjoowo et al., 2011).

A range of methods exist for detecting VBNC organisms. Vitality markers can be determined, e.g. the integrity of the cell membrane as determined by the live/dead-system, the presence of ribosomal RNA as an indicator for protein production or others. Table 1 shows a “toolbox”, compiled from Hammes and Egli, 2010; Rochelle et al., 2011 and Jungfer et al., 2013.

Organisms in the VBNC state can represent a significant and currently underestimated risk for the hygienic safety of drinking water. They are particularly relevant for drinking water installations in hospitals and other medical institutions, schools, retirement homes, kindergartens, army barracks, hotels, universities and other large buildings. Things become more problematic if immunosuppressed people are concerned and exposed to resuscitated pathogens.

Selected results from research project

Using spatio-temporal sampling strategies, the reliability of detection of microbial contaminations in drinking water...
installations of large buildings was investigated. It became obvious that a solid knowledge of the local conditions is crucially important. Also, in the same building, very different results were found at different sampling points. Even at the same sampling point, large variations were found during the course of the day. It is quite an achievement that a logistic regression model in combination with the potable hot water (PHW) constant temperature allows for a realistic assessment of the risk of a Legionella contamination (Völker et al., 2013). In a technical system, the efficacy of disinfection measures was investigated. Interestingly, P. aeruginosa seemed to be controlled by the autochthonic biofilm and regrowth had been promoted after disinfection measures which eradicated the autochthonic biofilm. Disinfection may offer a selection advantage to fast-growing pathogens, which are able to return particularly rapidly after disinfection. Copper induced the VBNC state in P. aeruginosa; this could be reversed by treatment with a copper chelator. After such treatment, infectivity was regained (Dwidjosiswojo et al., 2011; Flemming et al., 2013).

Acknowledgements

The results of the project are currently compiled in publications. The essence of the findings is available in German from the website of the project (biofilm-management.de) and will soon be translated.

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<table>
<thead>
<tr>
<th>Method</th>
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<th>Interpretation</th>
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<tr>
<td>DAPI, Syto 9, PCR</td>
<td>Nucleic acid</td>
<td>All cells, dead or alive</td>
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<tr>
<td>PI+Syto 9, PMA</td>
<td>Membrane integrity</td>
<td>Principally viable</td>
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<td>Rhodamin 123</td>
<td>Proton motive force</td>
<td>Energy conservation</td>
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<tr>
<td>Resazurin, CTC</td>
<td>Fluorescent resorufin, fluorescent formazan</td>
<td>Metabolic activity</td>
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<td>Oxonol (DiBAC₄)</td>
<td>Membrane penetration</td>
<td>Membrane potential</td>
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<tr>
<td>Fluorescein diacetate</td>
<td>Hydrolysis of FDA, fluorescein formation</td>
<td>Intracellular metabolic activity</td>
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<tr>
<td>rtPCR</td>
<td>Reverse transcriptase</td>
<td>Protein expression</td>
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<td>Direct viable count</td>
<td>Cell elongation</td>
<td>Growth sign</td>
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<tr>
<td>FISH, DVC-FISH</td>
<td>ribosomal RNA</td>
<td>Protein production</td>
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<td>ATP</td>
<td>ATP</td>
<td>Energetical state of cell</td>
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<td>Ethidium bromide</td>
<td>Expelling of EB</td>
<td>Efflux pump activity</td>
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<td>¹³C uptake</td>
<td>Cell-bound isotope activity</td>
<td>Assimilation, metabolic activity</td>
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Table 1: Toolbox for detection of viability markers of VBNC organisms
References


The Bunyonyi Impact Project

Bunyonyi Impact is a community-based project that supports health and education initiatives in the rural areas around Lake Bunyonyi, near Kabale. It was set up by Claire Kwesiga to offer better health and education services to the locals. The Lake Bunyonyi community clinic is the only clinic located in Rutinda and it strives to provide better health services for the people of Lake Bunyonyi. The aim of the project is to make health care available for the local residents, to improve their knowledge of family planning, and to provide antenatal/postnatal care and education on hygiene.

Making health care available to the local residents

The Mujera health centre II, located in Mujera, was built to serve local residents who are not able to go to the hospital in Kabale.

The health centre is visited by more than 30 outpatients a day. The most frequently treated diseases are gastrointestinal tract infections, respiratory tract infections, malaria and sexually-transmitted diseases. When patients visit the clinic, health workers take the opportunity to give them some health education. They teach patients about breast feeding, nutrition and preventable diseases, for example. In one case, for instance, health education was offered to a 38 year-old woman visiting the clinic for her ninth pregnancy. She had been previously tested as positive for HIV in the clinic. To protect the woman and her unborn child, health workers informed her during the visit about the possibilities of treatment and how to prevent her child from becoming infected. Educating patients about family planning is one of the main topics.

Improving knowledge of family planning and birth spacing.

It is not uncommon that a patient visiting the health centre for her ninth pregnancy is at the same time struggling with taking care of and feeding the eight children she already has.

Uganda’s total fertility rate at 6.2 is one of the highest both in sub-Saharan Africa and at global level (PRB, 2010). Maternal morbidity and mortality is high. According to Nalwadda et al. this can partly be attributed to unplanned pregnancies, short intervals between births and a higher risk of obstetric new-born complications associated with low contraceptive use (Nalwada, 2010). The aim of the project therefore is to inform patients about the possibilities of family planning. While providing information, it is important to find out what beliefs patients hold on family planning in order to debunk these myths. Patients in the clinic often think contraceptives cause permanent infertility, cancer or HIV.

Provision of prenatal care and postnatal care.

To improve the health of mother and child, the Majera health centre II provides antenatal and postnatal care to the local residents. The antenatal care includes pregnancy surveillance of the woman and her unborn child. The health workers examine the pregnant women to detect pregnancy-related complications such as preeclampsia, foetal problems and gestational diabetes. All the pregnant women are screened for underlying conditions and diseases, such as anaemia, malaria, sexually-transmitted infections, HIV infection, and underlying mental health problems and symptoms of stress or domestic violence. When health problems are diagnosed, either a suitable treatment is given locally, or the women are referred to the hospital in Kabale.

Immunization of children is of prime importance in postnatal care. The World Health Organisation claims that childhood vaccination prevents 2 million deaths per year worldwide. But there are still 2.5 million deaths a year caused by vaccine-preventable diseases, mainly in Africa and Asia among children under 5 years old. An immunization outreach program has been established to reach all mothers in the vicinity of the Majera health centre II. Health workers visit villages a 1 to 2 hour walk away from the health centre to immunize the children. During their visit, the babies are weighed to see how their growth curve is developing. If the weight is aberrant or they have other health problems, suitable advice or treatment will follow. In order to encourage mothers to attend all immu-
Health and hygiene education

According to the World Health Organisation, diarrhoea is the second leading cause of death among children under five. Pneumonia and diarrhoea account for nearly one-third of the deaths among children under five globally. Nearly 90 percent of deaths from pneumonia and diarrhoea occur in sub-Saharan Africa and South Asia. An important aspect of preventing diarrhoea is the improvement of drinking water quality, sanitation and hygiene. It has been estimated that 88 percent of diarrhoeal deaths worldwide are attributable to unsafe water, inadequate sanitation and poor hygiene.

The Bunyonyi Impact project educates the children of Hakahumiro primary school about the importance of hand washing with soap and encourages them to use clean drinking water.

Hakahumiro primary school has over 600 pupils; the school is located in Mujera, Lake Bunyonyi, Uganda. To improve sanitation hygiene, new toilets have been built for the children.

The toilets were sponsored by private individuals: Paul and Janet from Holland, Warwick and Chris from New Zealand. In these 'Ecological Sanitation' toilets, the urine and faeces are divided and no water is used. The underlying aim is to close nutrient and water cycles with as little as possible expenditure on material and energy, to contribute to a sustainable development. The nutrients contained in excreta are then recycled and can be used in agriculture.

The Bunyonyi Impact project keeps working hard for a healthy community free from preventable diseases and with better health services.

References


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Wetlands in Uganda – Ecosystems under pressure
A newspaper review from Uganda

“Without wetlands we cannot have water and without water we cannot have life and without life creation will cease to exist.” (Uganda’s Environment Minister Ephraim Kamuntu, Anguyo, 2013)

There is a high potential for conflicts over land use in areas providing pure water in certain countries in the world. A rapid change in the land use may have an important effect on the health of people settling in these areas. In Uganda, the struggle over the wetlands has reached a significant dimension since becoming a key topic in the newspapers. This newsletter article depicts the positions of the public discussion on the wetlands’ conflict in Uganda by reviewing three respected newspapers published in the time period from January 2013 to April 2014.

The importance of wetlands
Wetlands are swamps, banks, shores, floodplains, marshes, permanently-flooded areas, but also seasonally-flooded areas. Usually their soil is very fertile and they have a high biodiversity containing important bird areas. Wetlands store and filter polluted water. Thus they have an essential role in water collection and waste water treatment. Nine out of seventeen plant families identified in the wetlands around Kampala, for example, are used by the communities as healing plants (Daily Monitor, 03.04.2014). Furthermore, the wetlands provide nutrition such as agricultural products, fish, game and honey as well as materials such as fiber, herbaceous vegetation or space for tree planting. People send their livestock to the wetlands for grazing and also use the wetlands’ space to settle. Most of the people living around the wetlands are highly dependent on the environment (Wetlands Management Department et al. (ed.), 2009: 1).

Uganda is a land-locked country in Eastern Africa. It has a land area of 199,808 km² (Government of Uganda (GoU)) and 41,743 km² (GoU) of water bodies. 13 % of the land is covered by wetlands, which have a high value of ecosystem services (GoU). Since the government is aware of the important function of this fragile ecosystem, the wetlands in Uganda are governmentally protected areas, which should actually not be used for agricultural purposes. In charge of the implementation of laws on conserving wetlands is principally the Wetland Inspection Division (WID) together with the National Wetlands Programme (NWP). But the National Environment Management Authority (NEMA) also advocates the protection of wetlands, based on the 1995 national policy for the conservation and management of wetland resources (Huing, 2012).

Settlements within the wetlands
The increasing population pressure (Uganda’s population growth rate is 3.3%) and economic development, however, force people to settle in the wetlands and to use these areas for food production (Enenu, 2013). The landscapes are plundered and the biodiversity decreases rapidly. Extractions of the soil, which is common-
ly used for bricks, affect the biodiversity and deform the original topography (Timbiti, 2013). The production of bricks poses problems, not only due to the high demand for timber and the increasing carbon emissions, but also because the holes remaining from extractions become filled with water.

This stagnant water builds optimal habitats for mosquitoes and parasites, which transmit diseases such as malaria and schistosomiasis, and result in an increasing infestation of worms and parasites in the population. Moreover, the water quality is highly affected by the hard core mining which is widespread in wetlands, such as on the hills near Kigo Prison off Entebbe Road. “Dangerous chemical elements get washed down into the wetlands and subsequently into the lake, destabilizing fish breeding and the entire aquatic life” (Timbiti, 2013). Due to the high pressure, the landscape’s ecology is changing rapidly; this has crucial consequences on the availability of local food supply and the prevalence of water-related diseases in population.

Currently, the struggle about the wetlands is increasing with the controversy in the media over the role of government. People in place are supposed to leave the wetlands and settle in other places. Uganda’s President, Yoweri Museveni, mobilizes the Solicitor General in making people move from wetlands and claims the government’s ownership of the swamps. He threatens the population in place with actions in the case of wetlands degradation and encroachment (Otage & Ladu, 2014). The newspapers advise people on which areas they are allowed to settle and in which areas it is forbidden. They inform people about the environmental checks to run before building a house. “There is a recommended distance for people who want to build near a waterbody. This is because the lake’s water level changes according to the season and can result in flooding right up to the house. The protection zone or recommended distance from the lake not only protects the waterbody from pollution but also gives settlers time to flee in the case of dangerous floods […]” (Ndagire, 2014). In terms of environmental protection, the government’s actions are a positive progress stressing environmental advocacy.

Private investors claiming interests for the land

Meanwhile, in addition to the population on the ground settling in the wetlands areas, there is a whole bunch of stakeholders following their own interests. Land owners, for instance, earn money by leasing the land to hard-core miners and brick makers. Also, powerful businessmen and private investors claim a high interest in the wetlands (Daily Monitor, 03.04.2014) by making attractive offers to decision-makers. The wetlands around Kampala, for instance, have an essential function in water purification, since they filter 99.9% of the fecal matter per annum. But even these wetlands, like most of Uganda’s wetlands, “have been taken over by private developers who claim to have valid land titles” (Daily Monitor, 03.04.2014).

On the one hand, the investors bring money to the country and build shopping centres. This infrastructural change boosts the supply of imported food products and health facilities, which may contribute to the country’s development and to improving people’s living standards. On the other hand, this development of infrastructure and economy has resulted in massive construction work within the sensitive ecosystems in the last years. Today we can find hotels, shopping centres and nice private houses built in the wetlands. This ambiguous situation hampers the im-
The wetlands’ change and its impact on human health

It has not yet been investigated to what extent the use and the change of wetlands influences the water quality and the peoples’ life and health. The main expectations are the increase of bilharzia and malaria in static water areas, as found in rice fields. In addition, the loss of soil and unconstructed area reduces both the water storage and the filtering function of the wet-lands, thus lowering the water quality. Water that today is commonly used as drinking water can no longer be purified from bacteria and microbes. Thus higher incidences of diarrheal diseases can be expected. It seems that those people who are highly dependent on the wet-lands’ environment are most affected by changing these environments.

The NEMA and the Environment Ministry have the authority to recall land titles in protected areas (Daily Monitor, 07.03.2014), which promises progress in the protection of ecosystems. But it also means that every day people can be sent away from their houses. Thus many individual cases are affected by the struggle for land. Grant Kajura, who has lived here for six years, vividly remembers the Kigo swamp where residents grew crops and grazed animals. ‘People grew sweet potatoes and sugarcane here. We also fetched water, but when the place was filled with soil, they dug us a borehole so that we wouldn’t complain,’ Kajura says. ‘We first resisted the takeover of this place as residents, but we were overpowered.’ (Mwesigwa, 2013). The conflict generates a feeling of helplessness in the population. They feel at the mercy of decision-makers: ‘The people at the top will squeeze the small people if they touch the wetland, but leave the big people’, concludes Frank Muramuzi, the executive director of National Association for Professional Environmentalists (NAPE) (Mwesigwa, 2013). This permanent distress from struggles over the land and also a rapid change in the circumstances can cause mental diseases. Thus not only does the incidence of physical diseases increase, but there is also a high risk of an impact on mental health in the wetlands.

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Sophie-Bo Heinkel investigates the impact of converted wetlands on well-being in Uganda, considering place-bonding and place-making concepts. An emphasis in her work will be the motivation on protecting the landscape and using it in a sustainable way. Based on the RAMSAR Report 2012, the researcher is devising a literary review on the topic which she plans to publish next year.

References


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Events on Water, Health and Risk Communication

August

Small Water and Wastewater Systems National Conference
13-15 August
Newcastle (NSW), Australia

Water for Community Development and Prosperity
22-24 August
Kandy, Sri Lanka
http://www.communitywater.lk/

IAS Symposium on Activated Sludge: Past and Future 100 Years
26-28 August
Hongkong, China
http://iasprogram.ust.hk/201408/iasiwa/index.html

Stockholm World Water Week
31 August - 5 September
Stockholm, Sweden
http://www.worldwaterweek.org/

September

10-12 September
Essen, Germany
http://eps-perfect-slime-iwa.de/

IWA World Water Congress & Exhibition 2014
21-26 September
Lisbon, Portugal
http://www.iwa2014lisbon.org/

Summer school 2014: Advanced course on Resource Recovery from Wastewater
8-12 September
Ghent, Belgium
http://www.imetesummer.ugent.be/

October

IWA Regional Conference on Water Reuse and Energy 2014
21-24 October
Daegu, Korea
http://www.water2014.org/

3rd GRF One Health Summit 2014
5-8 October
Davos, Switzerland
http://onehealth.grforum.org/

November

12th IWA Specialist Conference on Small Water and Wastewater Systems & 4th IWA Specialist Conference on Resource Oriented Sanitation
2-4 November
Muscat, Sultanate of Oman
http://portal.cce.edu.om/iwa2014/

European Utility Week
4-6 November
Amsterdam, The Netherlands
http://www.european-utility-week.com/

7th meeting of the Working Group on Water and Health
25-27 November
Geneva, Switzerland

XII Latin American Workshop and Symposium on Anaerobic Digestion
25-28 November
La Habana, Cuba
http://www.11daal2014.com/

I3th meeting of the Bureau of the Protocol on Water and Health
28 November
Geneva, Switzerland

1st YWP Conference Germany: Advanced Wastewater Treatment and Water Reuse – The future is now!
11 June
Essen, Germany
http://www.iwahq.org/2bv/events/iwa-events/2014/ywp-conference-germany.html

December

1st Specialist Conference on Municipal Water Management and Sanitation in Developing Countries
3-6 December
Bangkok, Thailand
http://www.iwa2014ait.com/

4th meeting of the Implementation Committee of the Water Convention
4 December
Geneva, Switzerland

7th International Young Water Professional Conference
7-10 December
Taipei, Chinese Taiwan
http://www.iwa-ywp7.org/

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