Drink. Water Eng. Sci. Discuss., 5, 515–532, 2012
www.drink-water-eng-sci-discuss.net/5/515/2012/
doi:10.5194/dwesd-5-515-2012

Open Access Drinking Water Engineering and Science Discussions

Application of DVC-FISH method in tracking *Escherichia coli* in drinking water distribution networks

L. Mezule, S. Larsson, and T. Juhna

Department of Water Engineering and Technology, Riga Technical University, Riga, Latvia

Received: 1 September 2012 – Accepted: 12 September 2012
– Published: 26 September 2012

Correspondence to: L. Mezule (linda.mezule@rtu.lv)

Published by Copernicus Publications on behalf of the Delft University of Technology.

Abstract

Sporadic detection of live (viable) *Escherichia coli* in drinking water with molecular methods but not with standard plate counts has raised concerns about the reliability of this indicator in the surveillance of drinking water safety. Previous studies have shown that in low numbers metabolically active *E. coli* can be found in the biofilm of drinking water. The aim of this study was to analyse the distribution of non-cultivable *E. coli* in the drinking water distribution system meeting microbiological quality standards and evaluate the importance of the biofilm as its harbour.

In total 24 biofilm samplings and 40 at least 100 times pre-concentrated water samples were taken over a period of two years from two water treatment plants (surface water and groundwater) and four locations in water distribution network of a large city. Cultivable, total and able to divide (viable) *E. coli* concentration was measured in all samples.

The results showed that none of the network biofilm samples contained cultivable *E. coli* and less than two cells per 10 l were detected in the pre-concentrated water samples. However, almost all of the samples contained viable *E. coli* in the range of 1–50 cells per litre or cm^2 which represented approximately 53 % of all *E. coli* detected. The amount of viable *E. coli* was higher in the biofilm after surface water treatment when compared to the outlet from the groundwater plant and the number tended to increase from both treatment plants further into the network irrespective of the season.

In conclusion, *E. coli* in the water supply is not necessarily directly linked to recent faecal contamination and tends to accumulate in the networks where it is less exposed to disinfectants. Thus it can be accepted that biofilm formation in the drinking water distribution networks increases the risk of accumulation of viable but not cultivable *E. coli* in the system.

DWESD

5, 515–532, 2012

1 Introduction

Over the last century, monitoring of *Escherichia coli* by using standard plate count methods in water samples taken from several locations of water supply system (grab sampling) has allowed to increase the safety of public water supply system and significantly reduce the mortality caused by waterborne outbreaks in the developed countries. However, this strategy has not been always successful in detecting outbreaks, including several major ones (Lee et al., 2002). This approach has not also been able to explain sporadic contamination cases of drinking water (Hunter, 1997). There are several reasons for this bias including the following ones: (i) due to different rates of transport in aquatic environment and resistance to disinfection, the presence of *E. coli* does not always correlate with other pathogens (Ashbolt et al., 2001), (ii) some *E. coli* cells could be injured or stressed and unable to reproduce on growth media used for their detection (Scheusner et al., 1971) and (iii) the probability of detecting the contamination with grab sampling is low due to the small size of samples and limited sampling locations in the networks (van Lieverloo et al., 2007). The significance of the latter two factors in the context of understanding the fate of *E. coli* is only marginally studied.

Although it is still disputable whether so called viable but not cultivable (VBNC) state of bacteria actually exist (Bogosian et al., 1998), there are convincing evidences that not all alive (viable) *E. coli* are able to form colonies in the conditions used for standard plate counting (growth media, temperature, cultivation time) (Na et al., 2006). The importance of this bacterial subpopulation for public health is stressed by numerous molecular studies showing that they still retain ability of gene expression (Asakura et al., 2007; Oliver, 2010) including the ones responsible for pathogenicity (Pommepuy et al., 1996). In order to better understand the fate of *E. coli*, the application of molecular methods are needed which allow to detect microorganisms in different metabolic states including VBNC. Detection of bacteria which are able to elongate using, so called, direct viable count (DVC) (Kogure et al., 1979) in combination with fluorescence in situ hybridization (FISH) is one of the tools allowing to study VBNC bacteria.

DWESD
5, 515–532, 2012

In previous studies, to increase the probability of detecting *E. coli,* the composition of the biofilm was analysed (Juhna et al., 2007) The results showed that ca. 0.1 % from total microbial population in the biofilm consist of *E. coli* even though no indicators of faecal contamination were found by routine sampling and analyses of water. In the present study the sampling of biofilm and large volumes of water (concentrates) from the networks over the period of two years was performed and total, cultivable and VBNC (detected as DVC-FISH) cells were analysed to obtain comprehensive data about the distribution of *E. coli* in water distribution networks. With this approach it was expected to detect even a low number of *E. coli* and, hence, better understand the fate of these faecal origin bacteria in the networks. The study was carried out in a large water distribution network (Riga, Latvia) meeting drinking water quality standards with respect to microbiology.

2 Materials and methods

2.1 Site location and sampling

The total length of Riga drinking water supply system is 1374 km with a demand of 1500 l s^{-1}. The 76 % of all pipe length consists of cast and ductile iron, 19 % of steel and the rest of reinforced concrete and polyethylene (Rubulis et al., 2010). The left bank is supplied with two-stage treated surface water, whereas the right bank is supplied with groundwater treated by artificial groundwater recharge through infiltration. In both cases final chlorination is applied. Detailed parameters of the water can be found elsewhere (Tihomirova et al., 2010).

A total of 24 biofilm samples and 40 water samples were collected from five sites at water treatment stations and four sites in Riga drinking water supply system (Latvia) (Fig. 1). Points were located at surface and groundwater treatment plants before and during treatment and just after final chlorination (S-RW, S-DW, G-RW, G-DW and

DWESD
5, 515–532, 2012

G-DW′) and at four places in the network (S-NET1, S-NET2, G-NET1, G-NET2). Samples were collected over a period of two years and included all four seasons.

To collect the biofilm, a stainless steel biofilm collector containing five 3 mm diameter stainless steel coupons inserted into 25- to 40-mm pipes was attached to the distribution system and kept for two weeks to allow the biofilm formation. After two weeks the collectors were removed and immediately transported to the laboratory. Within two hours the biofilm was removed by ultrasonication for 2 min at 20 µA and 22 KHz. A total of 16–40 ml biofilm suspension in sterile distilled water was obtained and further analysed. All samples were analysed in double.

A large volume of water samples (more than 100 l) were concentrated to approximately 1 l using tangential ultrafiltration method. Total concentration time varied from 12–24 h. The apparatus used for concentration was similar to the one developed within TECHNEAU project (Veenendaal and Brouwer-Hanzens, 2007). The estimated recovery rate for the concentration of drinking water was 81 ± 33 %. The obtained concentrate was collected into sterile glass bottles, transported to the laboratory and analysed within two hours. All samples were analysed in triple.

2.2 Total bacterial numbers (TBN)

A known volume of sample was filtered onto 25-mm-diameter 0.2-µm-pore-size filters (Anodisc; Whatman plc), fixed with 3–4 % (v/v) formaldehyde and stained with 10 µg ml^{-1} DAPI (4',6-diamidino-2-phenylindole, Merck) for 15 min. Cell numbers were determined by epifluorescence microscopy by counting 20 random fields of view (Ex: 545 ± 30 nm; Em. 610 ± 75 nm, dichromatic mirror 565 nm, Leica DM, LB).

2.3 ATP measurements

ATP was determined according to a modified method described by Vital et al. (2008). In brief, 500 µl of pre-warmed sample were mixed with BacTiter-GloTM System (Promega, USA) and measured with luminometer. Results were expressed as ng ATP

DWESD
5, 515–532, 2012

per cm^2 of biofilm sample. For all samples total ATP and free ATP was measured and then cell ATP calculated. To obtain free ATP the sample was filtered through 0.1 µm pore size syringe filters (Sartorious, Minisart®, Germany).

2.4 Culture based methods

To obtain heterotrophic plate counts (HPC) samples were serially diluted in sterile distilled water and then inoculated onto R2A agar plates by spread plate technique. All plates were incubated in dark at 22 °C for 7 days. Results were expressed as colony forming units (CFU) per cm^2 (biofilm) or ml (water) of sample. For cultivable *E. coli* samples were analysed by membrane filtration method according to EN ISO 9308-1:2000 (ISO, 2001).

2.5 DVC-FISH

Cell viability-potential to divide, was determined by modified DVC method by Kogure et al. (1979) and combined with FISH. The validity of the FISH method used for detection of *E. coli* in this study has been proven earlier where it was compared with other molecular methods. In brief, samples were mixed with equal amount of Tryptone Soya broth (Oxoid Ltd., UK) and 10 µg ml^{-1} nalidixic acid and incubated for 6 h at 30 °C. After the incubation, samples were fixed with 3–4 % (*v*/*v*) formaldehyde for 20 min. Then the samples were filtered onto 25-mm-diameter 0.2-µm-pore-size filters (Anodisc; Whatman plc) washed with sterile distilled water, removed from filtration device and air-dried. Then, 20 µl of PNA hybridization mix consisting of hybridization buffer (50 mM Tris-HCl, 10 % *w*/*v* 50 % dextran sulphate, 0.1 mM of NaCl, 30 % *v*/*v* formamide, 30 % *v*/*v* tetra-sodium pyrophosphate, 0.2 % *w*/*v* polyvinylpyrrolidone, 0.2 % *w*/*v* Ficoll 400, 5 mM Na_2EDTA, 0.1 % *v*/*v* Triton X-100) and 200 nM fluorescently labelled PNA probe, was applied to the filters and covered with cover glass. The probe (TCA ATG AGC AAA GGT) (Perry-O'Keefe et al., 2001) was labelled with CY3 (Ex: 550, Em: 570) and flanked with solubility enhancers (Applied Biosystems, Foster City,

DWESD
5, 515–532, 2012

CA, USA) The samples were incubated at 57 °C for 60 min in a tight vessel in dark. After hybridization the filters were washed with plenty of sterile distilled water, air-dried and visualized with epifluorescence microscopy. Detection limits for *E. coli* was 6 cells per ml in concentrated water or 4 cells per cm^2 in the biofilm.

3 Results and discussion

A total of 86 waterborne outbreaks have been reported during 1990–2004 in 10 out of 25 European Union countries (Risebro et al., 2006). Both surface and groundwater supplies were identified as the possible sources of pollution, however, no statistical data exist about the number of cases related to the contamination though drinking water distribution network. During the last decade it has been recognised that the proportion of waterborne disease outbreaks associated with the distribution system failures are increasing (Moe and Rheingans, 2006). Thus, in this study sampling of water and biofilm was carried out from the water source to the tap, with a special emphasis on distribution networks.

During the last 10 yr, no waterborne outbreaks have been reported in a water supply system (Riga, Latvia) selected for the study. Analyses of raw water samples (river and groundwater) with standard methods (based on sample cultivation on growth media) showed that water sources contained less than four cultivable *E. coli* per 100 ml of sample (Table 1), which is an indication of relatively clean surface water. In contrast, DVC-FISH analyses showed that viable *E. coli* cell concentration was about 10 times higher. This is in agreement with previous studies about raw waters (Garcia-Armisen and Servais, 2004) which showed that only a fraction of alive *E. coli* are detected with traditional methods in raw water sources used for drinking water production.

Analyses of drinking water (after treatment and in the distribution network) showed that none of the water samples exceeded the standard value of zero cultivable *E. coli* in 100 ml. The applied sample concentration method allowed to decrease the detection limit when compared to traditional sampling methodology. The results showed that

DWESD
5, 515–532, 2012

about 30 % of water samples contained 0.02 to 1.2 cells in 10 l. All positive samples were obtained further in the networks (S-NET2, G-NET1 and G-NET2) rather than directly after water treatment. Hence, cultivable *E. coli* cells were present in drinking water, however, the concentration was very low to be detected with traditional sampling and analytical methodology. The analyses of water samples showed that the lowest amount of FISH positive cells was obtained from water samples (G-DW and S-DW) directly after the treatment plants (Table 1) and this number tended to increase further in the network. The same trend was observed also for viable *E. coli* (DVC-FISH positive) cells, where their concentration increased from few cells to more than 50 cells per litre as water proceeded into the networks.

The analyses of biofilm samples showed that at the treatment plant (S-DW) as much as 44 *E. coli* cells per cm^2 were present. This particular measurement could be biased by high background fluorescence intensity from humic substances present in the water (Tihomirova et al., 2010). However, the interference from autofluorescent background was overcome by analysing the viable cells with DVC-FISH as in this case the bacterial cells appeared very bright and visible due to their increased size (Fig. 2). The amount of viable *E. coli* in the biofilm at the treatment plant was 1.99 cells cm^{-2} (S-DW) and 0.66 cells cm^{-2} (G-DW) indicating that faecal bacteria sporadically can pass though the treatment plant and are harboured within the biofilm. In total the results of FISH analyses showed that each cm^2 of two week old biofilm contain a minimum of 5 *E. coli* cells (Table 1) and all 24 biofilm and 25 water samples were positive for *E. coli*. From all FISH positive *E. coli* in the biofilm, on average 53 % of cells showed the ability to divide.

Along with cell distribution trends, the influence on seasonality was analysed. Again, irrespective of the season, the highest amount of DVC positive *E. coli* was observed for points further in the network. Relatively more cells were observed in spring and winter seasons (data not shown); indicating that warmer outside temperatures are not linked to the occurrence of *E. coli* in the network, especially when the water temperature in the system is around 13 °C throughout the year.

DWESD
5, 515–532, 2012

The findings of faecal bacteria in the drinking water is not so surprising since previous research has shown that *E. coli* can survive in the drinking water biofilters (Li et al., 2006) and even multiply in the biofilm (Camper et al., 1991; Fass et al., 1996; Williams and Braun-Howland, 2003). Our study confirmed that the biofilm act as a source for accumulation and possible growth of *E. coli* in drinking water distribution networks. This should be taken into account when studying the fate of these bacteria from the source to the tap.

The highest amount of *E. coli* was found in the samples collected further away from both water treatment plants (Table 1). There was no correlation between cultivable and viable *E. coli* numbers in drinking water, unlike observed for raw water samples (Garcia-Armisen and Servais, 2004). Similarly the biofilm in locations further away in the network contained more *E. coli* cells with a higher percentage of DVC positive ones. The high numbers of *E. coli* could be due to their resistance to chlorine, low chlorine levels at all in the network or low protozoan grazing (Sibille et al., 1998). Their release from the biofilm by detachment (Parsek and Singh, 2003) can have a serious effect on human health, especially if VBNC forms of bacteria, including *E. coli* O157:H7 are present (Liu et al., 2009). Moreover, lately discussions about the existence of naturalized *E. coli*, although no pathogenicity was proven, raise concerns about secondary contamination of water supply systems due to pathogenic bacteria regrowth.

The particular water supply system is known to support bacterial growth due to the high level of labile organic carbon in drinking water (Tihomirova et al., 2010). Thus, biofilm formation was also studied. The analysis of coupon samples inserted in Riga drinking water distribution network showed that extensive biofilm formation occurred on surfaces just at the outlet from the treatment plant, reaching concentration about million cells per cm^2 (Table 2).

The amount of total bacteria, heterotrophic plate counts and *E. coli* in the biofilm tended to increase with the water residence time in the network. A positive correlation ($R^2 > 0.95$) between the total bacterial numbers and heterotrophic plate counts in the biofilm was obtained indicating on the formation of more favourable conditions

DWESD
5, 515–532, 2012

for microbial colonization and growth. Similar increase in the distribution network was also observed for water samples, where a correlation of $R^2 = 0.8869$ was obtained between HPC and TBN in five water samples from one sampling site. But, similarly as in previous studies, no correlation between total bacterial counts or HPC in biofilm and water was observed (Flemming, 2002). In water samples from both treatment plants the bacterial numbers tended to increase with the water residence time in the network. This has been observed previously (Juhna et al., 2007) and could be connected to decrease of disinfectant in the network in addition to increase of bacterial resistance and survival capacity (Gilbert and Brown, 1995; LeChevallier and Kwok-Keung, 2004; Saby et al., 2005). Thus, more favourable conditions for biofilm formation and maturing are created.

The results of ATP showed a high variation between the samples (from 1.1 till 2518 ng ATP ml^{-1}) and between the different seasons of samples from one location (7.35 till 2518 ng ATP ml^{-1}). The correlation between ATP and HPC or TBC was weak, which is similar to previous observations (Delahaye et al., 2007). Comparison of DVC positive *E. coli* with total bacterial counts showed a linear correlation ($R^2 = 0.9637$) in the biofilm, thus, indicating on the role of biofilm community on pathogen accumulation. Most of the studies that have examined the presence of *E. coli* in biofilms have used culture-based assays (Castonguay et al., 2006; Hu et al., 2005) which have limitations, including the duration of incubation, antagonistic organism interference, lack of specificity, and poor detection of slow-growing or non-dividing microorganisms (Rompre et al., 2002). Plate count methods also result in some inaccuracy since the cells can be clumped together and intertwined with other biofilm components. It must be emphasized that methods using microbial growth will not detect non-dividing cells at all. Therefore, the number of *E. coli* in the drinking water distribution network could be, and likely is, underestimated. The presence of *E. coli* was inadequately indicated by the traditional culture-based methods in the present study, a finding in agreement with previous studies showing that cultivation-independent detection methods detect at least 10 times more cells (Bjergbaek and Roslev, 2005).

DWESD
5, 515–532, 2012

4 Conclusions

The results of this study demonstrate the presence of viable *E. coli* in drinking water and biofilms even already after water treatment. Despite that both treatment processes are generally regarded as safe, some sporadic breakthrough of *E. coli* occur which lead to small numbers of *E. coli* missed in drinking water when analysed with traditional sampling methodology. Additionally, total and viable amount of *E. coli* tend to accumulate in the networks were it is less exposed to disinfectant. Thus, biofilm formation in the drinking water distribution networks increases the risk (at least during the first two weeks) of accumulation of viable but non cultivable *E. coli* and their presence does not necessary directly links to recent faecal contamination.

Acknowledgements. This work has been undertaken as a part of the research project "Technology enabled universal access to safe water – TECHNEAU" (Nr. 018320) which is supported by the European Union within the 6th Framework Programme. There hereby follows a disclaimer stating that the authors are solely responsible for the work. It does not represent the opinion of the Community and the Community is not responsible for any use that might be made of data appearing herein.

References

Asakura, H., Ishiwa, A., Arakawa, E., Makino, S., Okada, Y., Yamamoto, S., and Igimi, S.: Gene expression profile of *Vibrio cholerae* in the cold stress-induced viable but non-culturable state, Environ. Microbiol., 9, 869–879, 2007.

Ashbolt, N. J., Grabow, W. O. K., and Snozzi, M.: World health organization (WHO). Water quality: Guidelines, standards and health, Indicators of microbial water quality, edited by: Fewtrell, L. and Bartram, J., IWA Publishing, London, 2001.

Bjergbaek, L. A. and Roslev, P.: Formation of nonculturable *Escherichia coli* in drinking water, J. Appl. Microbiol., 99, 1090–1098, 2005.

Bogosian, G., Morris, P. J. L., and O'Neil, J. P.: A mixed culture recovery method indicates that enteric bacteria do not enter the viable but nonculturable state, Appl. Environ. Microbiol., 64, 1736–1742, 1998.

DWESD

5, 515–532, 2012

Camper, A., McFeters, G., Characklis, W., and Jones, W.: Growth kinetics of coliform bacteria under conditions relevant to drinking water distribution systems, Appl. Environ. Microbiol., 57, 2233–2239, 1991.

Castonguay, M. H., van der Schaaf, S., Koester, W., Krooneman, J., van der Meer, W., Harmsen, H., and Landini, P.: Biofilm formation by *Escherichia coli* is stimulated by synergistic interactions and co-adhesion mechanisms with adherence-proficient bacteria, Res. Microbiol., 157, 471–478, 2006.

Delahaye, E., Welte, B., Levi, Y., Leblon, G., and Montiel, A.: An ATP-based method for monitoring the microbiological drinking water quality in a distribution network, Water Res., 37, 3689–3696, 2003.

Fass, S., Dincher, M. L., Rasoner, D. J., Gatel, D., and Block, J.-C.: Fate of *Escherichia coli* experimentally injected in a drinking water distribution pilot system, Water Res., 30, 2215–2221, 1996.

Flemming, H. C.: Biofouling in water systems – cases, causes and countermeasures, Appl. Microbiol. Biot., 59, 629–640, 2002.

Garcia-Armisen, T. and Servais, P.: Enumeration of viable *E. coli* in rivers and wastewaters by fluorescent in situ hybridization, J. Microbiol. Meth., 58, 269–279, 2004.

Gilbert, P. and Brown, M.: Phenotypic plasticity and mechanisms of protection of bacterial biofilms from antimicrobial agents, in: Microbial biofilms, edited by: Lappin-Scott, H. E. and Costerton J. W., Cambridge University Press, 118–132, 1995.

Hu, J., Yu, B., Feng, Y., Tan, X., Ong, S., Ng, W., and Hoe, W.: Investigation into biofilms in a local drinking water distribution system, in: Biofilms, Cambridge University Press, Cambridge, 19–25, 2005.

Hunter, P. R.: Waterborne disease: Epidemiology and ecology, John Wiley and Sons, West Sussex, 1997.

ISO 9308-1:2000 Water quality – detection and enumeration of *Escherichia coli* and coliform bacteria – Part 1: Membrane filtration method, International Organisation for Standartization, 2001.

Juhna, T., Birzniece, D., Larsson, S., Zulenkovs, D., Sharipo, A., Azevedo, N. F., Me'nard-Szczebara, F., Castagnet, S., Fe'liers, C., and Keevil, C. W.: Detection of *Escherichia coli* in biofilms from pipe samples and coupons in drinking water distribution networks, Appl. Environ. Microbiol., 73, 7456–7464, 2007.

DWESD

5, 515–532, 2012

Kogure, K., Simidu, U., and Taga, N.: A tentative direct microscopic method for counting living marine bacteria, Can. J. Microbiol., 25, 415–420, 1979.
LeChevallier, M. W. and Kwok-Keung, A.: Water treatment and pathogen control: Process efficiency in achieving safe drinking water, IWA Publishing on behalf of the World Health Organization, London, 2004.
Lee, S. H., Levy, D. A., Craun, G. F., Beach, M. J., and Calderon, R. L.: Surveillance for waterborne-disease outbreaks – united states, 1999–2000, MMWR Surveill Summ, 51, 1–47, 2002.
Li, J., McLellan, S., and Ogawa, S.: Accumulation and fate of green fluorescent labeled *Escherichia coli* in laboratory-scale drinking water biofilters, Water Res., 40, 3023–3028, 2006.
Liu, Y., Wang, C., Tyrrell, G., Hrudey, S. E., and Li, X. F.: Induction of *Escherichia coli* O157:H7 into the viable but non-culturable state by chloraminated water and river water, and subsequent resuscitation, Environ. Microbiol. Rep., 2, 155–161, 2009.
Moe, C. and Rheingans, R.: Global challenges in water, sanitation and health, J. Water Health, 4, 41–57, 2006.
Na, S. H., Miyanaga, K., Unno, H., and Tanji, Y.: The survival response of *Escherichia coli* K12 in a natural environment, Appl. Microbiol. Biot., 72, 386–392, 2006.
Oliver, J. D.: Recent findings on the viable but nonculturable state in pathogenic bacteria, FEMS Microbiol. Rev., 43, 415–425, 2010.
Parsek, M. and Singh, P.: Bacterial biofilms: An emerging link to disease pathogenesis, Annu. Rev. Microbiol., 57, 677–701, 2003.
Perry-O'Keefe, H., Stender, H., Broomer, A., Oliveira, K., Coull, J., and Hyldig-Nielsen, J.: Filter-based PNA in situ hybridization for rapid detection, identification and enumeration of specific micro-organisms, J. Appl. Microbiol., 90, 180–189, 2001.
Pommepuy, M., Butin, M., Derrien, A., Gourmelon, M., Colwell, R., and Cormier, M.: Retention of enteropathogenicity by viable but nonculturable *Escherichia coli* exposed to seawater and sunlight, Appl. Environ. Microbiol., 62, 4621–4626, 1996.
Risebro, H., de France Doria, M., Yip, H., and Hunter, P.: Intestinal illness through drinking water in Europe. Microrisk final report. Microbiological risk assessment: A scientific basis for managing drinking water safety from source to tap. Quantitative microbial risk assessment in the water safety plan, EVKI-CT-2002-00123, 1, 2006.

DWESD
5, 515–532, 2012

Rompre, A., Servais, P., Baudart, J., de-Roubin, M., and Laurent, P.: Detection and enumeration of coliforms in drinking water: Current methods and emerging approaches, J. Microbiol. Meth., 49, 31–54, 2002.
Rubulis, J., Dejus, S., and Meksa, R.: Online measurement usage for predicting water age from tracer tests to validate a hydraulic model, 12th Annual Water Distribution Systems Analysis Conference, Arizona, Tucson, Tucson (USA), 2010.
Saby, S., Vidal, A., and Suty, H.: Resistance of Legionella to disinfection in hot water distribution systems, Water Sci. Technol., 52, 15–28, 2005.
Scheusner, D. L., Busta, F. F., and Speck, M. L.: Inhibition of injured *Escherichia coli* by several selective agents, Appl. Microbiol., 21, 46–49, 1971.
Sibille, I., Sime-Ngando, T., Mathieu, L., and Block, J. C.: Protozoan bacterivory and *Escherichia coli* survival in drinking water distribution systems, Appl. Environ. Microbiol., 64, 197–202, 1998.
Tihomirova, K., Rubulis, J., and Juhna, T.: Changes of nom fractions during conventional drinking water treatment process in Riga, Latvia, Wat. Sci. Technol., 10, 157–163, 2010.
van Lieverloo, J. H., Mesman, G. A., Bakker, G. L., Baggelaar, P. K., Hamed, A., and Medema, G.: Probability of detecting and quantifying faecal contaminations of drinking water by periodically sampling for *E. coli*: A simulation model study, Water Res., 41, 4299–4308, 2007.
Veenendaal, H. R. and Brouwer-Hanzens, A. J.: A method for the concentration of microbes in large volumes of water in, D 3.2.4, TECHNEAU, 1–30, 2007.
Vital, M., Hammes, F., and Egli, T.: *Escherichia coli* O157 can grow in natural freshwater at low carbon concentrations, Environ. Microbiol., 10, 2387–2396, 2008.
Williams, M. M. and Braun-Howland, E. B.: Growth of *Escherichia coli* in model distribution system biofilms exposed to hypochlorous acid or monochloramine, Appl. Environ. Microbiol., 69, 5463–5471, 2003.

DWESD
5, 515–532, 2012

Table 1. Total cultivable, FISH positive and DVC positive (viable) *Escherichia coli* in the biofilm and water samples. Values are the average for four seasons with standard deviation specified as a maximum and minimum interval.

Sampling site	Sample type[a]	Cultivable *E. coli*, cfu cm^{-2} or cfu l^{-1}[b]	FISH positive *E. coli*, cells cm^{-2} or cells l^{-1}	DVC positive *E. coli*, cells cm^{-2} or cells l^{-1}
S-RW	W	26.74	n/d	$(2.52 \pm 0.17) \times 10^3$
	B	0	44.21 ± 34.72	1.99 ± 2.54
S-DW	W	0	n/d	0
	B	0	8.40 ± 3.92	2.95 ± 5.14
S-NET1	W	0	n/d	0.93 ± 1.61
	B	0	111.58 ± 57.87	45.20 ± 30.25
S-NET2	W	0.13	n/d	9.30 ± 4.13
G-RW	W	30.24	n/d	$(2.99 \pm 0.94) \times 10^3$
	B	0	9.39 ± 1.67	0.66 ± 0.85
G-DW	W	0	n/d	1.01 ± 1.75
G-DW′	W	0	n/d	0
	B	0	14.44 ± 4.94	3.54 ± 4.79
G-NET1	W	0.06	n/d	0.94 ± 1.62
	B	0	70.62 ± 19.97	84.75 ± 57.37
G-NET2	W	0.006 ± 0.01	103.97 ± 20.05	52.41 ± 58.72

Values are the average for four seasons with standard deviation specified as a maximum and minimum interval.
[a] W – water, B – biofilm
[b] For biofilm samples the results are represented as cfu or cells per cm^2, for water samples – cfu or cells per Liter of water
N/D – not determined

DWESD
5, 515–532, 2012

Table 2. Total bacterial numbers (TBN) and heterotrophic plate counts (HPC) in the biofilm samples.

Sampling site	TBN, cells $cm^{-2} \times 10^6$	HPC, cfu $cm^{-2} \times 10^4$
S-DW	5.64 ± 7.62	4.04 ± 5.33
S-NET 1	3.17 ± 3.88	8.40 ± 11.89
S-NET 2	17.13 ± 24.90	41.51 ± 21.62
G-DW	1.12 ± 0.48	0.78 ± 1.44
G-NET 1	7.71 ± 10.35	3.14 ± 2.71
G-NET 2	28.97 ± 18.14	178.50 ± 49.82

Values are the average for all biofilm samples tested during four seasons with standard deviation specified as a maximum and minimum interval.

DWESD
5, 515–532, 2012

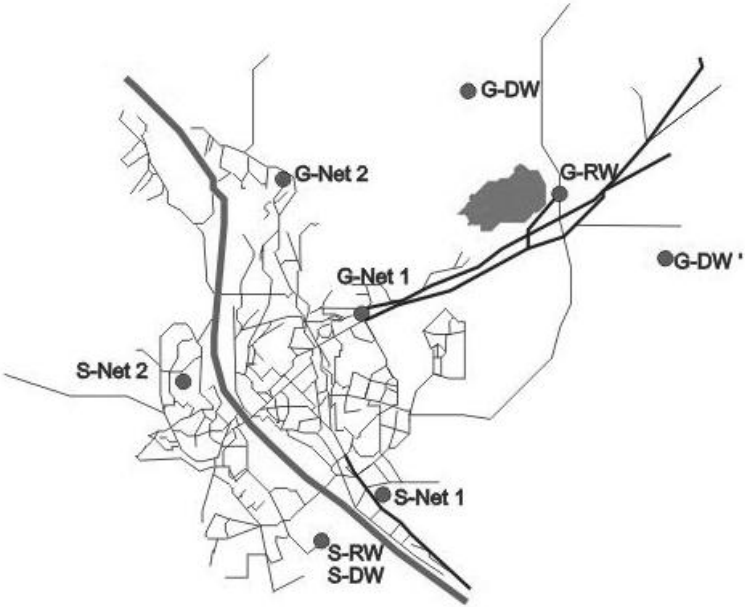

Fig. 1. Riga drinking water distribution network with 9 biofilm and water sampling sites including water before (G-RW – raw lake water; S-RW – raw surface water) and after treatment (S-DW (surface water after final chlorination) and, G-DW (infiltrated groundwater after final chlorination) and in the network S-NET1, S-NET2, G-NET1 and G-NET2. Numbers 1 and 2 indicate the residence time after treatment – "1" having shorter residence time.

DWESD
5, 515–532, 2012

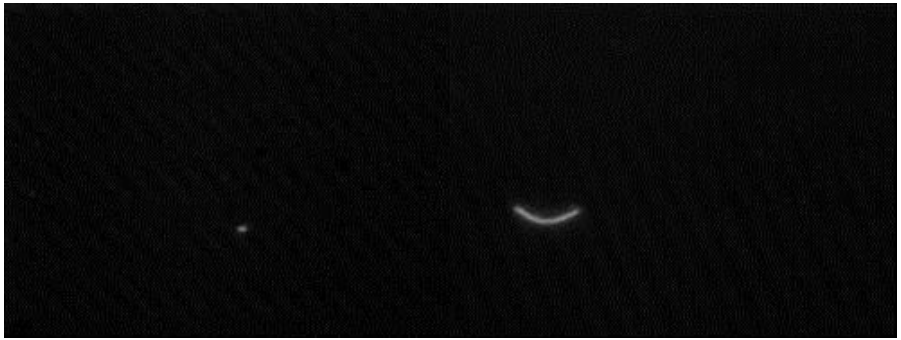

Fig. 2. G-DW biofilm sample obtained in winter season with FISH positive *E. coli* (left) and DVC-FISH positive *E. coli* (right).

DWESD
5, 515–532, 2012