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Drinking Water Engineering and Science Discussions

Interactive comment on "Application of DVC-FISH method in tracking *Escherichia coli* in drinking water distribution networks" *by* L. Mezule et al.

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We thank Mr. Azevedo for comments and specific questions addressed.

Apart from the authors interpretations, what I believe that can be taken from the manuscript (or at least more deeply discussed) is that the accumulation of viable but not cultivable E.coli might be acceptable at a certain level (yet to determine) in bioïňĄlms and at a lower extent in suspension and not bring any water safety concerns to consumers. At least this is in agreement with the fact that no outbreaks were observed during the time that the study took place. What are the authors views on this matter? How reliable is the surveillance of the waterborne outbreaks? Would it be possible in the future to indicate a threshold concentration for which the VBNC E. coli presence might cause disease in humans?

Based on reported data and our previous experience we can conclude that the occurrence of these E. coli is not always linked to the occurrence of human infections, what could arise the doubts about suitability of E. coli as faecal indicator. Moreover, the knowledge about the existence of both pathogenic and non-pathogenic forms of E. coli arise concerns on the nature of identified non-cultivable E. coli. To assess the direct effect of these identified VBNC E. coli their isolation and subsequent analysis must be performed. Unfortunately this was not possible in this study. Due to this, at the moment it is impossible to indicate any potential background concentration of VBNC in drinking water. However, it might be an issue in the future. The outbreak registration in Latvia is mostly limited to major outbreaks, which have not occurred. However, there is no information about occurrence of sporadic infections, since relatively mild diarrheal cases are not usually reported. Thus, we can only accept that there have been no cases of infections with pathogenic forms of E. coli, e.g. O157. This explanation will be introduced in the manuscript. Page 521, line 15: ....the last 10 yr, no major waterborne outbreaks have been reported. . .

For the size of the network, it might be advisable to increase the number of sampling sites in future studies, or else select a subsection of the network. This would allow for an easier interpretation and correlation of the different parameters, and a better control of the system under study. Yes, that might give a more detailed picture on the occurrence and transport. Here we wanted to include as many different sampling sites as possible – untreated, treated water, different residence time in the network.

SpeciïňĄc comments: Page 519, lines 13-14: "The estimated recovery rate for the con-centration of drinking water was 81 33 %". How was this estimated? The recovery rates were taken from the report of Veenendaal, H. R., and Brouwer-Hanzens, A. J . which was included in the reference list. In our studies this type of device was used. For accuracy the reference in the text will be relocated: Page 519, lines 12-14: The apparatus used for concentration was similar to the one developed within TECHNEAU project. The estimated recovery rate for the concentration of drinking water was 81 $\pm$

33% (Veenendaal and Brouwer-Hanzens, 2007). The obtained concentrate was...

Page 519, line 22: Please give more details on the model of the microscope and separate the parts related to the microscope ïňĄlters. Only one ïňĄlter was speciïňĄed. Was the PNA probe signal detected in the same ïňĄlter as the DAPI signal? A correction will be made: page 519, line 22: (Ex: 340/380 nm;. Em: >425 nm, dichromatic mirror 565 nm, Leica DM, LB) instead of (Ex: 535 $\pm$ 25 nm; Em. 610 $\pm$ 37 nm, dichromatic mirror 565 nm, Leica DM LB). On Page 521, line 3 the following explanation will be added: ... microscopy (Ex: 535 $\pm$ 25 nm; Em. 610 $\pm$ 37 nm, dichromatic mirror 565 nm, Leica DM LB).

Page 521, line 4: How were the detection limits assessed? Are there any references or experimental work supporting those values? The detection limits were calculated based on volume of analysed sample, repetitions and microscope fields counted. The counting technique to obtain these detection limits is described in more detail in: Mezule L.: Significance of Nonculturable Escherichia coli in Drinking Water: Experimental and Pilot Studies in Large Drinking Water Systems, Lambert Academic Publishing, 2012.

I would like to see some statistics on the manuscript. Are differences between results statistically meaningful (for instance, when comparing between seasons or samples from different locations). Single factor ANOVA was performed for FISH and DVC-FISH results obtained during all seasons at different sampling sites. The results showed that for DVC-FISH data there was no significant difference between the values ($p>0.05$). For the FISH counts significantly different results were obtained only in 2 sampling sites – S-DW ($p<0.02$) and S-NET1 ($p<0.05$) where in winter and spring months respectively higher total E. coli counts were observed. Respectively, for these samples high variance between the repetitions was observed. These explanations will be inserted in the manuscript in the results and discussion part.

Interactive comment on Drink. Water Eng. Sci. Discuss., 5, 515, 2012.