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Drinking Water Engineering and Science Discussions

Interactive comment on "Correlations between total cell concentration, total adenosinetri-phosphate concentration and heterotrophic plate counts during microbial monitoring of drinking water" *by* E. Siebel et al.

E. Siebel et al.

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The critical comments and suggestions by the reviewer are appreciated. Below are the answers to the specific issues that were raised. Comments posted previously are repeated as to present the final reply in one package.

(1) Use of alternative methods (Q-PCR, Microscopy)

The reviewer suggests the use of additional alternative methods such as Q-PCR and microscopic counting. Unfortunately the former technology was not available at the time of study, though a comparison on sensitivity, cost and ease-of-use would be in-

teresting. Microscopic counting is a usual part in the flow cytometer calibration. If the counting is performed accurately, the difference between these two methods is less than 10 % (although microscopic counting usually gives higher standard deviation). The problem with microscopic counting is that it can be extremely time consuming, though this can be overcome with some automated microscopes (e.g. Pernthaler et al., 2003, AEM 69: 2631)

(2) Should water guidelines change ?

The reviewer questioned how results of the new methods, showing much higher cell numbers than conventional HPC data, should be interpreted in terms of the drinking water guidelines? This links to the comment of Reviewer 2 regarding the limitations of the study. This most simple answer is that much more comparative data are needed which cover as many different types of water and treatment scenarios before new guidelines can be established and/or old methods are discarded. What we have observed so far is that for non-chlorinated drinking water the following general values are observed:

- Total cell concentrations (TCC) are typically between 1 - 3 x 10ˆ5 cells/mL in tap water produced from surface water

- TCC are typically between 0.5 - 2 x 10ˆ5 cells/mL in tap water produced from groundwater

- TCC are typically between 0.5 - 3 x 10ˆ5 cells/mL in bottled drinking water

The most logic opinion would be to use both TCC and conventional HPC in a complimentary manner for the time being. It is also important to note that TCC would provide incomplete information in cases where disinfection (e.g. chlorine residuals) is used. Hence, the combination with viability parameters such as ATP analysis becomes more important. Were possible, we have addressed these points in the amended version of the manuscript.

(3) Several fundamental questions on ATP

REVIEWER: What is the effect of cell cycle and growth rate on ATP level per cell?

Author comment: Growth rate has indeed an important impact on ATP levels in bacterial cells. For example, when cells from a natural microbial community are grown in batch, the cellular ATP level peaks at early exponential phase at levels about 3X higher than the stationary phase values (unpublished data from our group). This is, however, not an increase in the cellular ATP concentration as such, but rather correlates with increased cell biovolume (larger cells contain more ATP). Hence, a logic use of ATP would probably be to correlate it to active biovolume in a sample (see also comment for Reviewer 2). It should be noted that bacteria in tap water samples are probably in a semi-starvation stage, more similar to stationary phase cells, hence, the effect of growth rate might be low.

REVIEWER: Which fraction of ATP originates from intact cells, and which fraction is dissolved in the bulk water?

Author comment: For the tap water samples in this study, the free ATP (measured after 0.1 um filtration of selected samples) from the water can be neglected ($<$ 5%). However, as we have shown previously (Hammes et al., 2008), this can be quite significant and should be check in water samples. We have added this to the revised manuscript.

REVIEWER: Is the fraction of culturable cells stable?

Author comment: No, this fraction is not stable. If the fraction of culturable cells was stable, one would have observed a good correlation with TCC (Table 1), which was not the case.

(4) What is known about the water ?

REVIEWER: On p.77 line 14, regrowth is mentioned as a reason for increase of cell count in the distribution system. Is anything known about the finished water of the treatment plant to prove this point?

Author comment: Indeed, the water from the treatment plant and distribution system has been measured previously (Hammes et al., submitted; Berney et al., submitted). As both studies are still unpublished, we have included the relevant data as such (unpublished data) in the revised manuscript, and have also elaborated a bit on this point.

(5) Technical corrections

The comments are appreciated and the corrections have been made in the revised manuscript.

Interactive comment on Drink. Water Eng. Sci. Discuss., 1, 71, 2008.