Aquavalens Project

"Protecting the health of Europeans by improving methods for the detection of pathogens in drinking water and water used in food preparation."

Report on the microLAN system performance

- short report on the performance in river water

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Introduction

Monitoring of drinking water quality is an important and necessary task in order to protect public health. Enteric pathogens are the pathogenic bacteria most frequently found in drinking water. Since it is not possible to analyze drinking water for all important pathogens, so called indicator organisms are often used as surrogates. Coliform bacteria, which all belong to the taxonomic family *Enterobacteriaceae,* are present in large numbers in the intestinal tract of humans and other warm blooded animals and are therefore often used as indicator organisms for faecal contamination in drinking water. Although they are also frequently found in natural environments, drinking water is not their natural habitat and their presence is an indication for a possible threat or impairment of water quality. The coliform *E. coli* is the most important indicator for recent faecal contamination in drinking water. It only originates from the intestinal tracts of warm-blooded animals, where it is the most common coliform bacterium and does not propagate in drinking water (Stevens et al. 2003). Along with other coliforms, *E. coli* should not be present in 100 ml drinking water according to the European drinking water directive (Council Directive 98/83/EC).

The group of coliform bacteria shows a broad diversity in terms of genus and species. The current definition of coliforms is essentially based on shared biochemical characteristics. One of them is the possession of the enzyme β-D-galactosidase (GAL), which is needed for metabolizing the sugar lactose. Currently, more than 15 bacterial genera are recognized as coliform bacteria by this definition (Stevens et al. 2003). According to the European drinking water directive, two methods for the detection and enumeration of *E. coli* and coliform bacteria in water can be used. One of them is the DIN EN ISO 9308-1 which uses membrane filtration and cultivation on Chromocult Colifom Agar (CCA), the other DIN EN ISO 9308-2, a MPN method called Colilert®-18/Quanti-Tray® (Colilert) which is marketed by IDEXX Laboratories. Both methods are based on cultivation along with enzymatic reactions for identification and allow a simultaneous detection of coliform bacteria and *E. coli*. While all coliform bacteria are GAL positive, *E. coli* additionally possesses the enzyme β-D-glucuronidase (GLUC).

Since a cultivation step is needed for the detection of coliform bacteria and *E. coli* with CCA and Colilert, final results take up to 24 h to be reported. Additionally to the cultivation duration, time for sampling and sample-processing should be taken into account. In the last decades, the development of online-monitoring devices for drinking water emerged. While chemo-physical and hydrological water quality parameters are already frequently monitored by such devices, no reliable online monitoring devices for microbiological parameters are standardized for permanent use as alternatives to cultural methods.

An automated system detecting coliform bacteria and *E. coli* semi-continuously based on the enzymatic activities had been developed by the former Aquavalens partner mbOnline. During the runtime of the Aquavalens project, the company microLAN took over this system from mbOnline and became the subsequent partner within Aquavalens. The device, now called BACTcontrol, allows a detection of elevated coliform- and *E. coli* numbers in water within approximately 2.5 hours. Like the culture-based methods DIN EN ISO 9308-1 and DIN EN ISO 9308-2, BACTcontrol relies on the activity of the enzymes GAL for coliform bacteria and GLUC for *E. coli*, respectively, however without implementing a cultivation step. The aim of this Deliverable was the report on the microLAN system performance regarding the detection limit and specificity of BACTcontrol compared to the traditional cultural methods.

Measurements of natural water samples

In order to analyze the enzymatic activity of coliform bacteria and *E. coli* in natural water samples, water samples were taken from the River Rhine and a karst water source and were measured with the BACTcontrol device for GLUC and GAL activity. Additionally, the MPN of *E. coli* and coliform bacteria was determined by Colilert in parallel.

River Rhine

Figure 1 shows the results of the measurements of river water of the river Rhine over 6 months (December 2015 – June 2016). The detection limit for coliform bacteria and *E. coli* originating from these river water samples seems therefore to be between 10^1 and 10^2 cells/100 ml. Consequently, reliable results with the enzymatic activity measurements with BACTcontrol are expected at cell counts above 10^2 cells/100 ml.

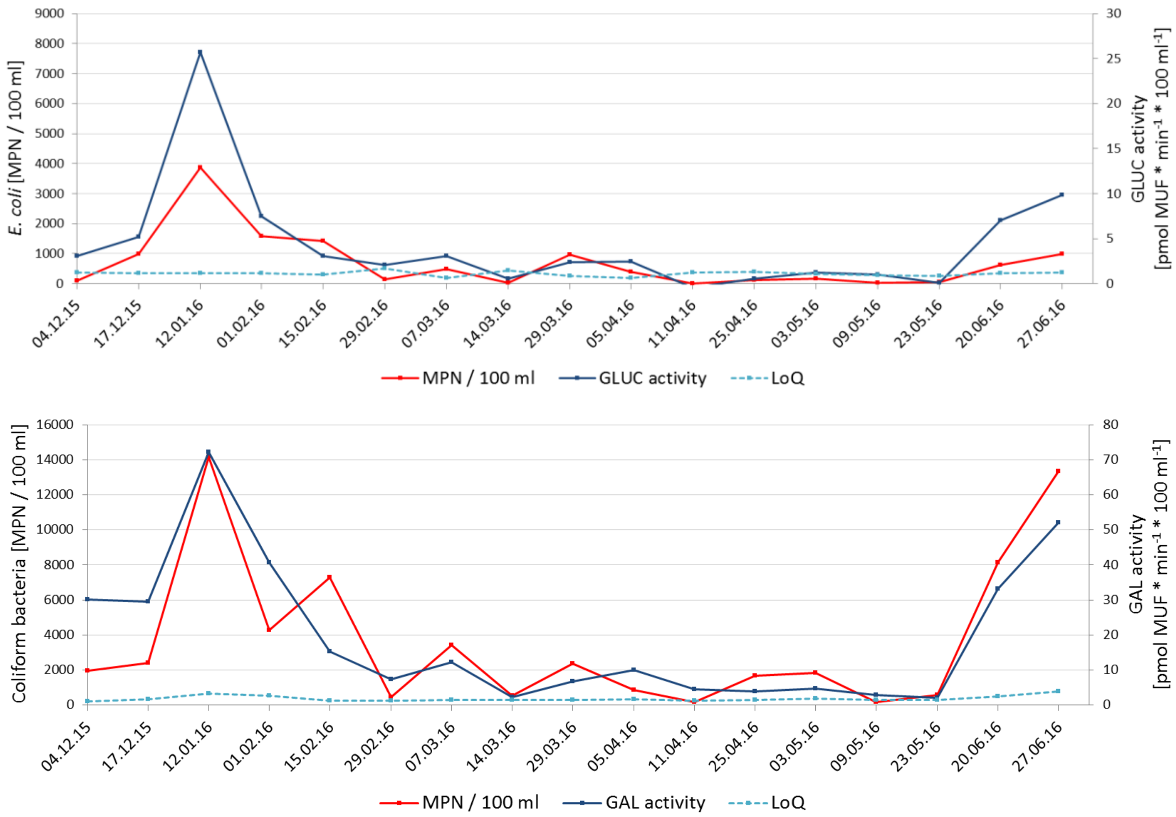

Figure 1: Results of the analysed river water samples taken from December 2015 – June 2016. The enzymatic activity in the samples was measured with BACTcontrol and compared to the cultural method using Colilert. Above: GLUC activity and detected MPN of *E. coli* per 100 ml. Below: GAL activity and detected MPN of coliform bacteria per 100 ml. The respective LoQ was calculated for each measurement by BACTcontrol.

Taken the results together, there is an obvious correlation between detected cell numbers of coliform bacteria and *E. coli* with Colilert and BACTcontrol. High MPNs resulted generally in elevated enzyme activities.

Discrepancies between rapid enzymatic assays and cultivation methods have been observed previously. In these field trials, enzymatic assays yielded a high enzymatic response with a low recovery on cultivation methods which was explained by false positive results and viable but non-culturable (VBNC) cells (George et al. 2001).

VBNC bacteria are bacterial cells with a detectable metabolic function but without cultivability by using available cultural methods. Bacteria are known to be able to shift into that metabolic state when they are subjected to environmental changes like unbeneficial nutrient condition, salinity, osmotic pressure, temperature and pH (Colwell 2000). It further has been shown that VBNC bacteria could maintain virulence und could be therefore of importance for the evaluation of human risk consuming different kind of waters (Colwell et al. 1985, Grimes, Colwell 1986). Since these VBNC bacteria could still contain or even produce the enzymes GAL or GLUC, it is possible, that they are detected by BACTcontrol but not with the cultural methods.

In the present study, however, in some cases decreasing enzymatic responses were accompanied by increasing numbers of bacteria detected with the cultural methods which cannot be explained by false-positive results or the occurrence of VBNC bacteria. Not only the environment but also the bacterial condition like the permeability of the cell membrane, the cell composition and the bacterial cell size could affect the induction of enzyme production in addition to the nutritional status of the cells. Furthermore, different bacterial strains could express different levels of GAL or GLUC, thus leading to enzyme levels depending on the composition of bacteria. Thus, it is not possible to perfectly correlate actual cell numbers with measured enzymatic activity.

Conclusion

Coliform bacteria including *E. coli* should not be present in 100 ml of drinking water. Their detection is usually performed with two culture-based methods, namely DIN EN ISO 9308-1 which uses membrane filtration and cultivation on CCA or, alternatively, Colilert®-18/Quanti-Tray® (DIN EN ISO 9308-2). Since these cultural methods both need up to 24 hours to give a result, online-devices like the BACTcontrol system from microLAN which detect coliform bacteria and *E. coli* in less than 2.5 hours are of considerable interest to the drinking water sector.

One big advantage of the BACTcontrol system, besides the short detection time, is the possibility to directly connect the device to the water source which can then be analyzed fully automatically. BACTcontrol could therefore be used as an early warning system for emerging coliform bacteria and *E. coli* cell counts in drinking water or for monitoring of water quality of raw water sources for drinking water production as well as recreational waters or water for agriculture and aquaculture.