Importance

Biofilms are organized in highly efficient and stable ecosystems and play a major role in the sorption of indicator bacteria as well as microbial pathogens. The importance of biofilm processes has long been recognized and a lot of studies investigating the fate of model pathogens have been carried out. Drinking water utilities commonly use coliform bacteria such as *Escherichia coli* as an indicator of the presence of pathogens from faecal contamination. Frequent occurrences of increased coliform numbers in the distribution system without a contamination event point towards that biofilms on the surfaces of pipes and fittings may act as a reservoir for coliforms and other pathogenic bacteria. The survival and cultivability of E. coli in water distribution networks are influenced by many environmental factors. It is therefore important to examine the behavior of this microorganism in a controlled system which mimics the situation in the distribution system (temperature and flowthrough).
Chlorination is a common way of drinking water disinfection, applied in many countries. However, despite the worldwide use of chlorine for disinfection of drinking water, water-mediated disease outbreaks occur again and again. In drinking water treatment, the inactivation of microorganisms increases with increasing disinfectant exposure (product of concentration and contact time, Ct). Thus the use of Ct (concentration of disinfectant in mg/L times, time in minutes) values is considered to be one of the options for controlling pathogens in drinking water. As it has been pointed out that chlorine disinfection might be one of the factors altering the physiology of a bacterium into an active but not cultivable state, culture based methods are perhaps not the most suitable ones for Ct value determination.

Approach

The behavior of *E. coli* in a controlled system was studied using Propella™ reactors, containing synthetic water and inoculated with approx. 10^7/mL of cells. The experiments were performed non-aseptically thus mimicking the natural conditions of a water distribution pipe where a sudden contamination has occurred. Total cell count was performed with DAPI; *E. coli* cells were enumerated using fluorescence in situ hybridization (FISH) and quantitative real time (QRT)-PCR assays. Cell viability was monitored using a culture based method (TBX medium) and direct a viable count (DVC) method in combination with FISH. Respiratory activity was measured using the 5-cyano-2,3-ditolyl. tetrazolium chloride (CTC) method. The influence of chlorine on the cultivability of *E. coli*, the ability to divide and on the respiration was also investigated in batch conditions (pH 7, 20ºC) applying 0.54 mg/l of chlorine and monitoring the reaction for up to 180 minutes. Samples were taken at different time points and the reaction was subsequently stopped by the addition of sodium thiosulphate. The cell viability was monitored using a culture based method (TBX medium) and direct viable count (DVC) method in combination with FISH. Respiratory activity was measured using the CTC method.

Results

Our experiments in the bioreactor showed that even if the cultivability is lost relatively rapidly but the ability to divide is still retained (indicated also by the growth rate of 0.03 h^{-1}) as well as the respiratory activity. Both the CTC and the DVC method

were tested and found to be reliable. QRT-PCR was used to confirm the data obtained by the FISH method and both datasets showed a good correlation.
An interesting correlation in the biofilm *E. coli* cells was observed, namely that the distribution of the cell length became narrower with time which was already obvious after 3 hours of the experiment. There was, however a noticeable difference in the cell number and the extent of cell enlargement comparing the initial and the last sample. This difference was investigated further by calculating the normal distribution coefficient for each of the 300 cells for each sample. A correlation was obtained, which could be used to assess the physiological state of cells, even their residence time in the drinking water, provided that sufficient number of cells are counted. In the presence of chlorine *E. coli* lost cultivability before the respiratory activity and, finally, the ability-to-divide was lost which is an indication that culture-based methods should not be the only analysis tool used for assessment of drinking water safety. For viability assessment both the DVC and the CTC methods should be used, because depending on the disinfection method either DVC or CTC positive cells could survive longer. Ct values (mg l^{-1} min^{-1}) were 0.2 for cultivability, 1.1 for respiration and 1.0 for the ability to divide.

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