THE MOLECULAR DETECTION FOR THE VIABILITY OF YERSINIA CELLS DURING SHOCK CHLORINATION – A PILOT SCALE STUDY

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1 INTRODUCTION

Efficient decontamination of pipeline system is an essential action in situations where a drinking water distribution system is contaminated. The challenge in decontamination is the fact that biofilms may protect faecal and pathogenic microbes against disinfectant after contamination event.[1,2] The one of the most frequently used cleaning method for drinking water distribution networks is clorination.[3,4,5,6] Shock-chlorination can be used for purification of microbial contaminated water distribution system in waterborne outbreaks. As an example, shock-chlorination was applied to clean the network after the severe waterborne outbreak in the Nokia city, Finland year 2007.[5,7]

It is also important to detect pathogenic microbes from water and biofilm samples as fast and efficient as possible if suspected contamination. The rapid and specific detection techniques for pathogenic bacteria could save valuable time and may reduce illness cases during an emergency situation. It also enables the verification of the success in the cleaning procedure.[8]

The new molecular biological techniques allow detection of viability of target pathogens as combination of Propidium monoazide (PMA)[9] treatment with quantitative polymerase chain reaction (qPCR) and Direct Viable Count (DVC) cell elongation with Peptide Nucleic Acid probes and Fluorescence in situ Hybridization (PNA-FISH).[10] Moreover, the traditional cultivation could take several days to weeks for reporting final results[6] when PCR and FISH based assays can provide results faster.[8,11,12] In addition, the FISH and PCR based techniques have been reported to be able to observe pathogenic bacteria from water and biofilm samples when the traditional cultivation method was not able to detect them.[13, 8]

The aim of this study was to test how a pilot water distribution network can be cleaned after contamination with bacteria using shock-chlorination.

2 THE EXPERIMENTAL SET-UP

The pilot distribution system consisted of a 400m long PEX plastic pipeline. Water flow in the system was 1.8 l/min. The quality of water and growth of the biofilms was followed for a period of one month prior the contamination phase. The progress of decontamination was

followed with the chlorine concentration, DAPI staining and heterotrophic plate counts (HPC). The chlorine concentration used for shock-chlorination was 10 mg Cl_2/l. During the experiment two parallel outlet water and three biofilm samples were took in each sampling point.

The experiments were carried out using *Yersinia pseudotuberculosis* strain, the closest relative surrogate bacteria for severe human bacterial pathogen *Yersinia pestis*. The effect of shock-chlorination for microbiological agents was followed with new molecular biology techniques such as PMA-PCR and PNA-DVC-FISH assays for the determination of viability of the surrogate bacteria. A photoreactive PMA compound cannot pass through the cell membrane of a viable bacterial cell. When a cell membrane is damaged, the PMA gets into the cell and covalently binds to the cell genome. The attached PMA inhibits real-time PCR reactions which allow observing differences between viable and damaged cells.[9,14] The combination of DVC-FISH technique includes enrichment of samples in the presence of an antibiotic.[10,15] The antibiotic prevents normal cell division leading to elongation of viable cells.[16] A fluorescent PNA probe can bind as a sequence specific for ribosomal RNA of target cells. The successful binding between the PNA probe and specific target can microscopically be observed. When the fluorescent elongated cells could be detected the bacterial viability state can be estimated.[17] In addition, the *Yersinia* selective cefsulodin-Irgasan-novobiocin (CIN) plate counting was used as parallel with the new molecular biological techniques for detection of cultivable *Yersinia* cells.

3 RESULTS

The concentration and viability of *Yersinia pseudotuberculosis* cells decreased rapidly in water and biofilm samples after start of the chlorination (Figure 1 and 2) as well as the counts of heterotrophic plate count and total microbial counts. In biofilms, the FISH counts of *Yersinia* were reduced by approximately 5 log and with qPCR 4 log.

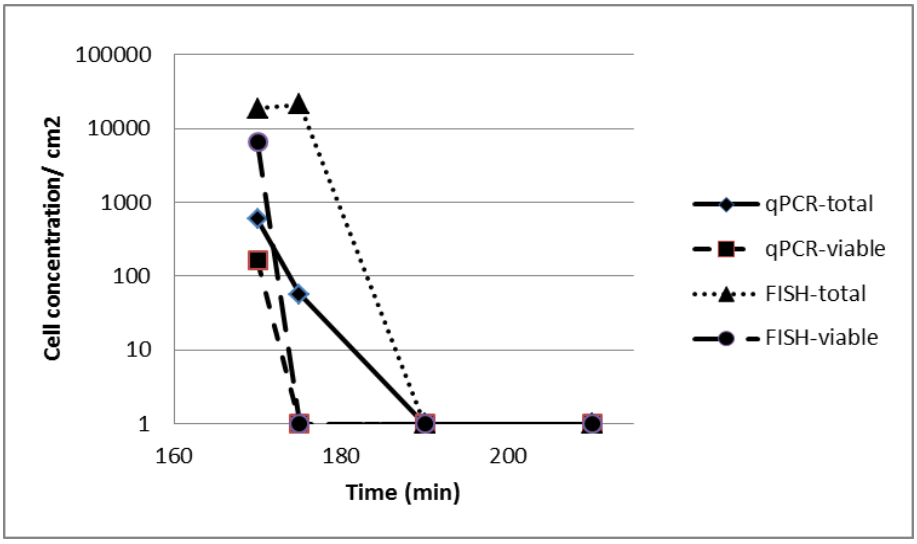

Figure 1 *The viable/ VBNC and total cell concentration in biofilm samples.*

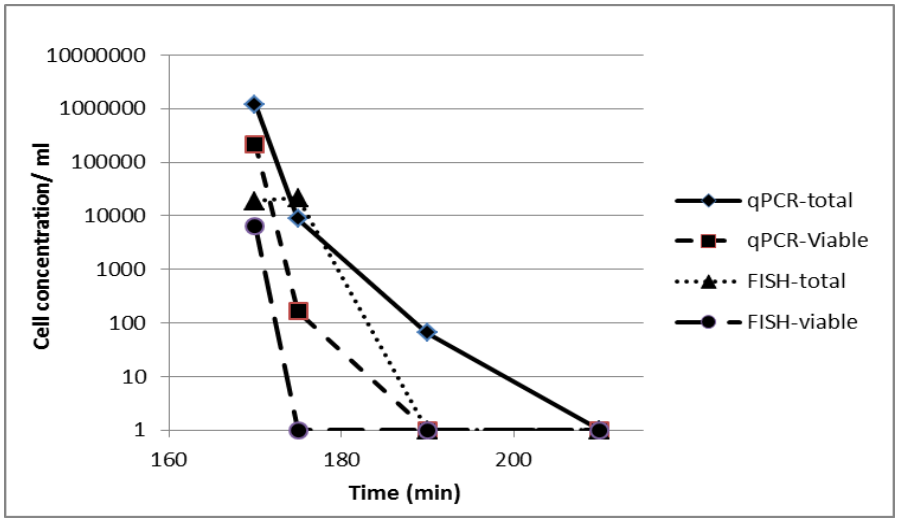

Figure 2 *The viable/ VBNC and total cell concentration in water samples.*

In water, the FISH and qPCR counts of *Yersinia* were reduced by approximately 7 log. The new PCR and FISH techniques were able to detect *Yersinia* cells (total counts/ viable counts) while the selective culture method could not found any vegetative bacterial cells.

4 CONCLUSIONS

In this study it was found out that the new qPCR and FISH based detection techniques were usable for determination of viability of the *Yersinia pseudotuberculosis* cells. According to previous studies, it has been stated that traditional FISH and PCR techniques could not separate live, viable but non-cultivable (VBNC) or dead cells from live culturable cells and that conventional cultivation based methods should be used for the detection of live, cultivable bacterial cells.[6,18] It is known that chlorine damages nucleic acids[19] which may affect the results of nucleic acid based FISH and qPCR methods. Overall, this study indicated that the shock-chlorination appeared to be an effective technique for cleaning a water distribution network after a bacterial contamination. However, it is obvious that some of the *Yersinia* cells introduced in the pipeline may have washed away from the pipeline network during the experiment. This might explain part of the rapid decrease in *Yersinia* cells in the water and biofilm samples. Therefore it is difficult to assess the true inactivation efficiency of shock-chlorination for the removal of the microbial contaminant.

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