Separation of bacteria from loose deposits of drinking water distribution systems

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Abstract

During drinking water distribution unwanted loose deposits tend to accumulate in distribution systems. Loose deposits originate from pipe material corrosion as well as from the particles and flocks entering system from the treatment plant. These deposits may provide substrate for and serve as carriers of bacteria (Le Chevallier et al., 1987; Torvinen et al., 2004;). Direct counting using fluorescence microscopy is a common method used to evaluate bacterial abundance in many natural systems. Enumeration of sediment samples presents a substantial challenge due to the heterogeneity of the samples, adhered bacteria and increased background fluorescence due to high abundance of inorganic particles. The association between microbial cells and the sediment matrix requires the use of specific extraction procedures to allow efficient recovery of cells from sample materials. Such techniques for the detachment of bacteria from particles have mainly been optimized for soil samples (Epstein and Rossel, 1995; Whiteley et al., 2003; Maron et al., 2006). The aim of this study was to develop a method for separation of bacteria from loose deposits found in drinking water distribution systems. Several techniques based on centrifugation were tested in the laboratory scale. *Escherichia coli* and *Bacillus subtilis* were chosen as model organisms for Gram-negative and Gram-positive spore-forming bacteria. Cells were mixed with wet loose deposits and the bacteria were then separated by using high or low speed centrifugation with or without density gradient. The fraction containing microbial cells was then filtered through 0.22 μm pore size polycarbonate filter, stained with DAPI (4',6-diamidino-2-phenylindole) and enumerated using the epifluorescence microscopy. At least 20 fields or at least 300 bacteria were counted in triplicate samples.
E. coli cells were separated from the deposits more efficiently compared to the vegetative cells of *B. subtilis*. The high speed centrifugation using a density gradient medium was more effective compared to the centrifugation without density gradient or at low speed (recovery rates of 33% and 7% (*E. coli*), 10% and 5% (vegetative cells of *B. subtilis*), respectively).

Keywords: loose deposits, bacteria, detachment, density gradient

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