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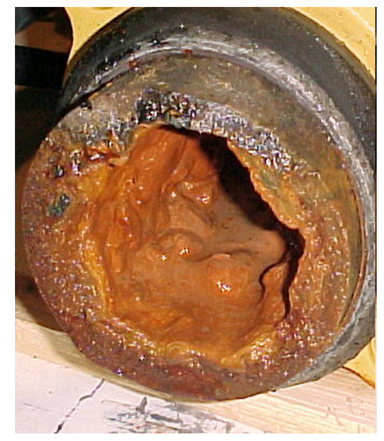

Live/Dead Discrimination of Biofilm Bacteria from a Drinking Water Pilot Distribution System

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MOTIVATION

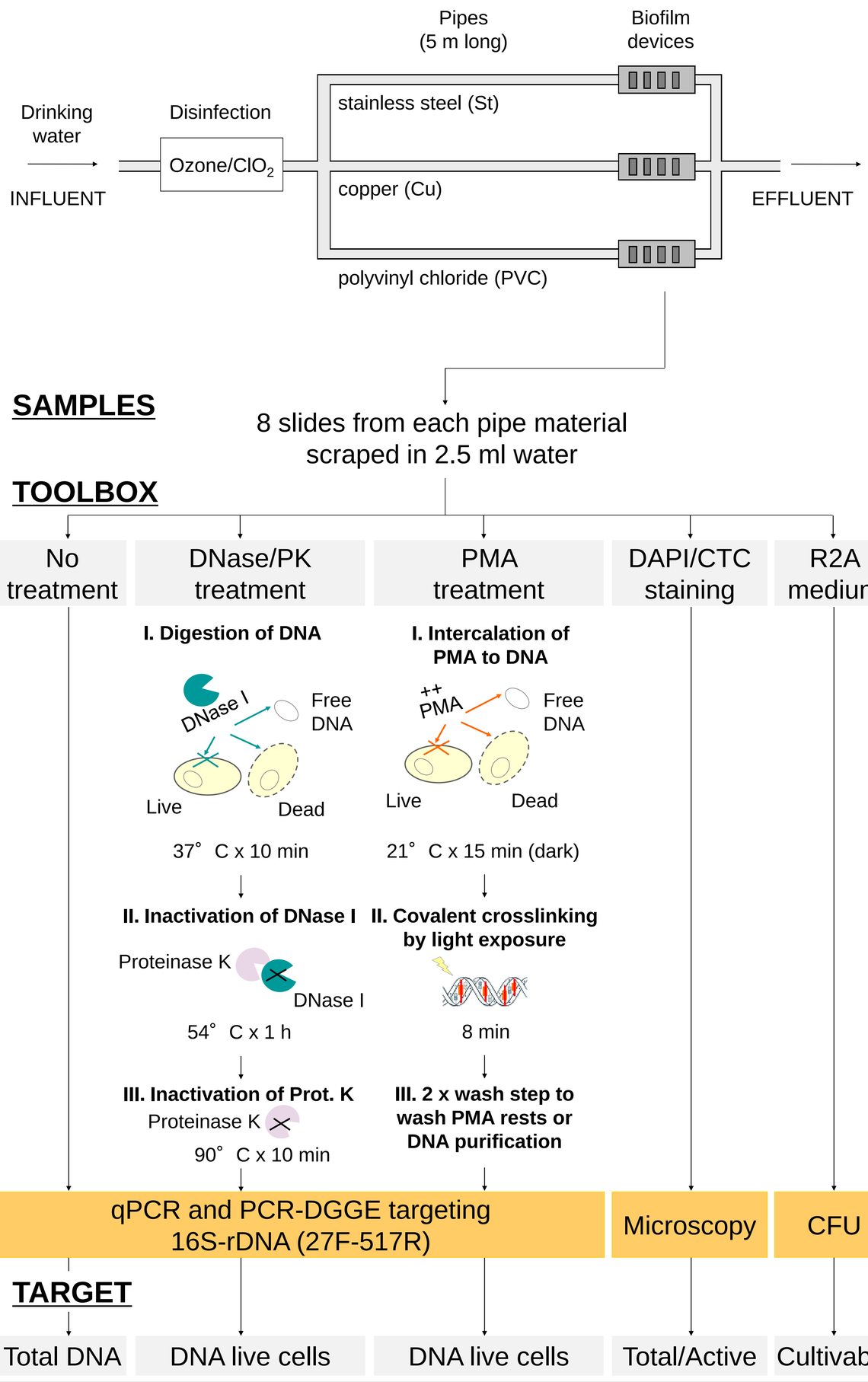
Formation of biofilms in drinking water distribution networks, including plumbing systems, are of great concern. Biofilms are potential habitats for all kinds of bacteria, including pathogens, and may be responsible for contaminations of bulk water systems.

Nowadays, DNA-based methods are used for the detection and characterization of bacteria. One of the major disadvantages of these techniques is that they cannot distinguish between DNA from live and dead cells. A battery of methods to face this problematic is presented in this work.

MATERIALS AND METHODS

Conditioned surface water disinfected with ozone/ClO_2 flowed through a pilot scale of a distribution system for biofilm formation. Biofilm bacteria were analyzed using a molecular biology toolbox.

PILOT SYSTEM



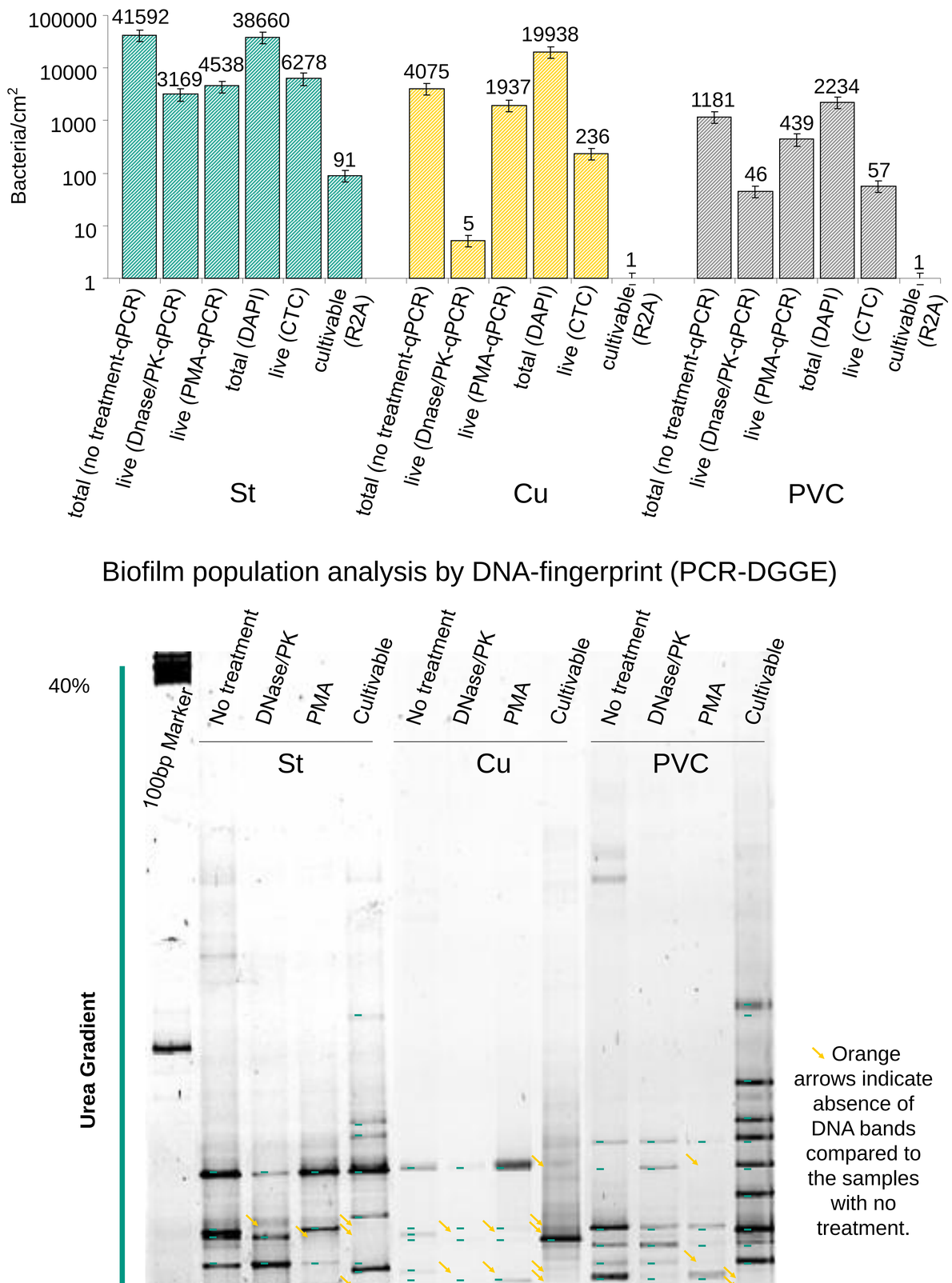
CONCLUSIONS

Using the live/dead differentiation toolbox for the analysis of natural drinking water biofilms it was possible to discriminate and quantify total, live, and cultivable bacteria.

PCR-DGGE analysis and the quantification methods demonstrated: (i) the applicability of PMA and DNase/PK treatment; (ii) DNA from dead bacteria and eDNA was blocked or digested by treatment with PMA or DNase/PK; (iii) DNase/PK treatment demonstrated a more distinct effect on live/dead differentiation; and (iv) the difference observed in the DGGE patterns indicated that the materials where the biofilms grew might have had an effect on the balance between live and dead bacteria present in the biofilms.

RESULTS

Quantitative detection (qPCR, microscopy, and CFU) of total, live, and cultivable cells in biofilms



Biofilm population analysis by DNA-fingerprint (PCR-DGGE)