Biofilm formation on model surfaces of drinking water distribution system

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INTRODUCTION

Biofilms are structures made of extra polymeric substances which harbour bacteria forming complex matrices and are ubiquitous also in aquatic environments, including drinking water distribution systems (DWDS). Numerous publications show that pathogenic microorganisms may be present in drinking water, so drinking water can also pose a food safety risk. In some cases they may contain non-pathogenic but opportunistic ones like *Pseudomonas aeruginosa.*

Aims of this work were to investigate (1) biofilm formation of tap water microbiota on High Density Polyethylene (HDPE) surface, and the (2) biofilm formation of two *Pseudomonas aeruginosa* strains on HDPE surface under different temperature conditions.

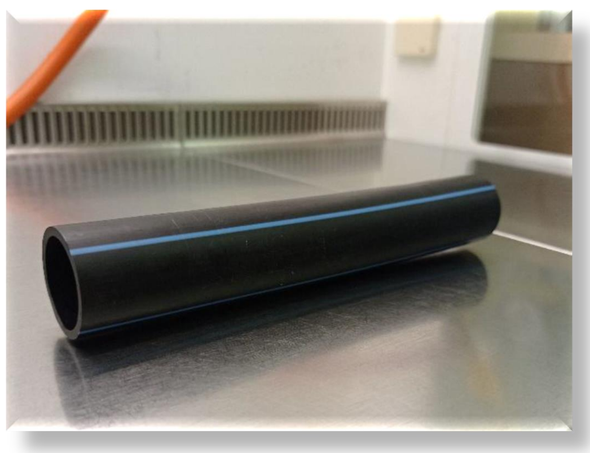

Figure 1. HDPE pipe commonly used in Drinkig water distribution systems.

MATERIAL AND METHODS

Biofilms were formed first on High Density Polyethylene (HDPE) surfaces in non-sterilized tap water at 25°C . HDPE pipe surfaces were applied to simulate the drinking water distribution pipes mostly and commonly used around the Globe (**Figure 1**). The pipes were cut into sizes of approximately 13.5 cm^2, cleaned and sterilized before use. This study was conducted in sterile environments under a laminar flow chamber. Throughout the investigaton the biofilm formation of the tap water microbiota and the layout of the model HDPE surfaces were studied. In a small bottle filled with 160 ml of non-sterilized tap water the model HDPE was placed in diagonally, in a big bottle containing 300 ml non-sterilized tap water and in one Petri dish with 30 ml of non-sterilized tap water the HDPE sufaces were placed in horizontal position (**Figure 2**). On days 0, 2, 5 and 8 of incubation the microbial concentration of the water and the surfaces was determined by traditional culturing methods.

Figure 2. Layout of the HDPE model surfaces

In order to test the biofilm formation ability of a model microorganism on HDPE surface the same investigations were carried out in R2A broth at 8 °C, 15 °C and 30 °C, respectively, with two different *Pseudomonas aeruginosa* strains: one of them previously isolated from DWDS and another originating from culture collection. The subsequent sampling days were separated by 24 and 48 hours, 7, 10 and 14 days.

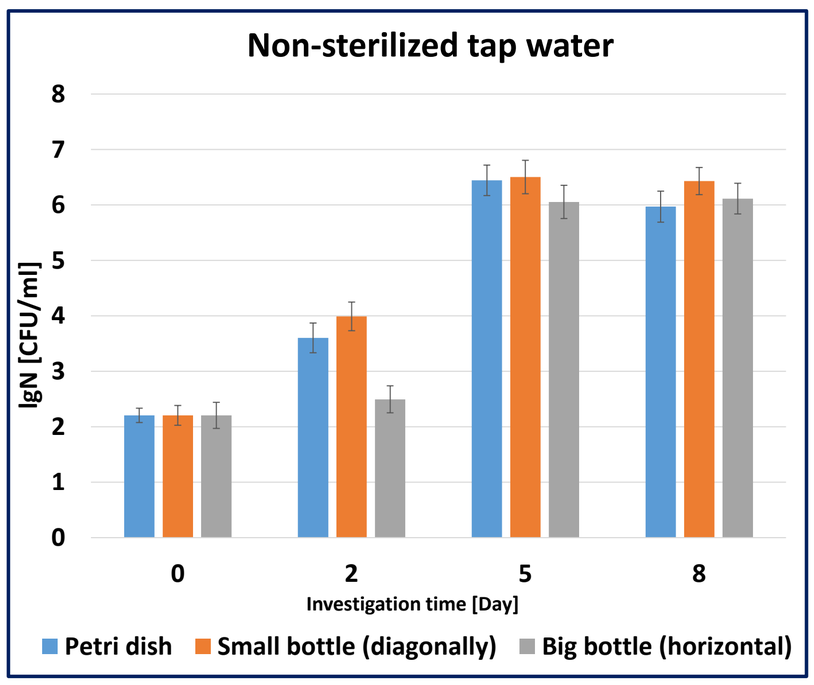

Figure 3. Changes of microbial concentrate in non-sterilized tap water

RESULTS

Figure 3. shows the changes in microbial concentration in the non-sterilezed tap water. Between days 2 and 5, the number of microbes increased significantly, several orders of magnitude. No significant differences can be observed among the vessels used for the study in terms of changes in the microbe.

Figure 4. shows the changes in the microbial concentration in the biofilm formed on the surface of the HDPE pipe pieces placed in three different containers. Throughout the study, it was found that the location of the pipe pieces in the tap water does not effect the biofilm formation, however the most microbes were detected on the surfaceses placed in the small glass, but the difference compared to the others is not remarkable.

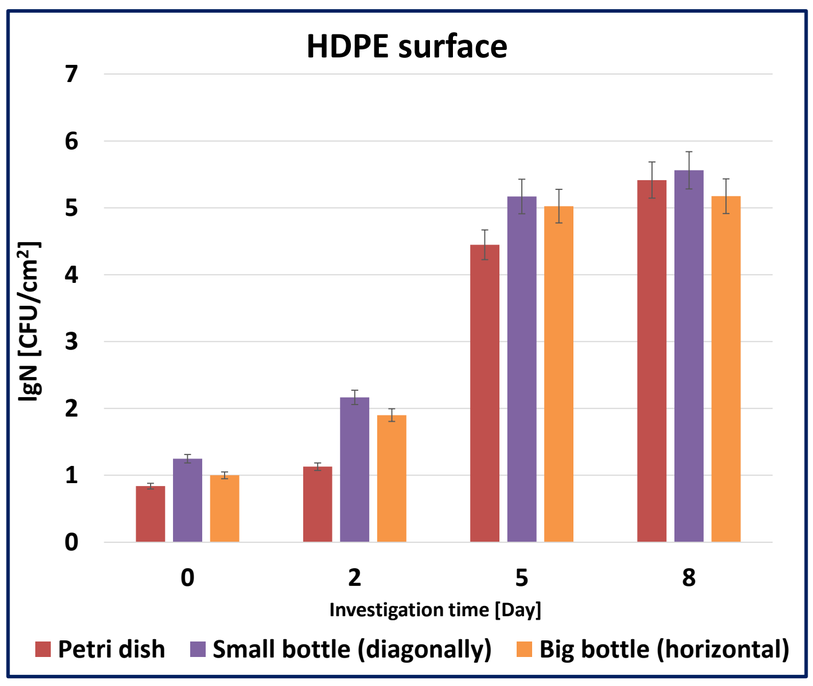

Figure 4. Changes of microbial concentrate on HDPE surfaces

Overall initial cell concentration in tap water increased intensively in the first days of incubation to 5x10^6 cfu/ml. Concentration of irreversible attached cells on the HDPE surface was initially 10 cfu/cm^2 that increased in 8 days 4 log cycle.

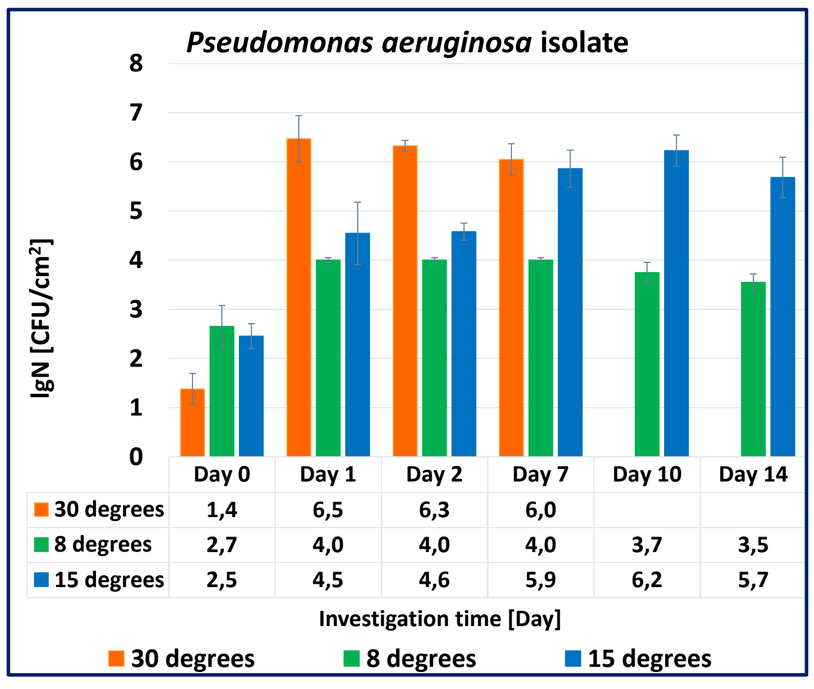

Figure 5. Temperature effect on biofilm formation by *Pseudomonas aeruginosa* isolate

In the course of the second experiment the biofilm formation of two *Pseudomonas aeruginosa* strain were studied. In **Figure 5** it is evident that temperature had a clear and direct effect on the growth of Pseudomonas aeruginosa isolate biofilms, with the higher the temperature, the higher the growth of the biofilm.

In **Figure 6** after 7 days, there was the highest biofilm formation of the ATCC 9027 strain at 15 ^{0}C which was the same as the highest at 30 ^{0}C recorded after 48 hours. Both observed accelerated development at high temperatures (>30°C), progressive growth at medium temperatures (15°C), and reduced biofilm growth at low temperatures (8 °C). Both the *Pseudomonas aeruginosa* isolate and the ATCC 9027 strain were significantly influenced by the temperature treatment.

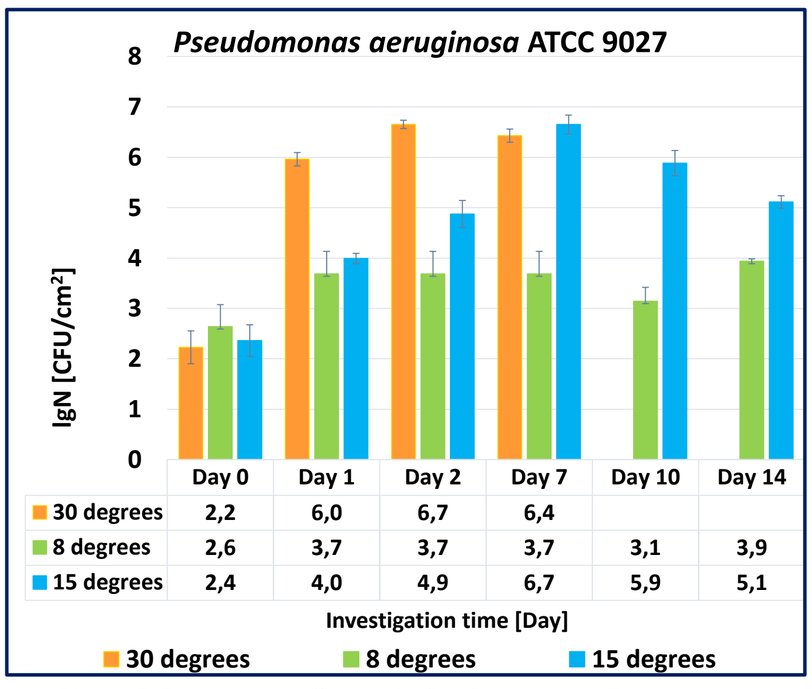

Figure 6. Temperature effect on biofilm formation by *Pseudomonas aeruginosa* ATCC 9027

Biofilms by *Pseudomonas aeruginosa* in drinking water distribution HDPE pipe surfaces increased prolifically at 30 ^{0}C, had a suppressed growth rate at 8 ^{0}C and a gradual increasing growth at 15 ^{0}C. It was clear that elevated temperatures promote biofilm growth while low temperatures inhibit their growth.

ACKNOWLEDGEMENT

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