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Biofilm formation in drinking water distribution systems.

Biofilms are structures made of extra polymeric substances which harbour bacteria forming complex matrices. They are ambiguous in drinking water distribution systems and some cases may contain pathogenic bacteria or non-pathogenic but opportunistic ones like *Pseudomonas aeruginosa.*

This study focused on the ability of the *Pseudomonas aeruginosa* bacteria to form biofilms in drinking water distribution systems. Several studies have been conducted but few focused on this opportunistic bacterium. Two of its strains *Pseudomonas aeruginosa* isolate from tap water and a strain from culture collection (ATCC 9027) were used.

The key objectives of this study were to determine the temperature and chlorine effects on the biofilm formation by *Pseudomonas aeruginosa* and compare those effects between the *Pseudomonas aeruginosa* isolate and ATCC 9027 strains.

Two treatments of temperature and chlorine were administered to assess their effects on biofilm growth. 30 ºC, 8 ºC and 15 ºC were used for the temperature treatment to simulate how this bacterium would form biofilms across different weather seasons that is, summer, winter, and spring/autumn, respectively.

0.1mg/l and 1mg/l chlorine concentrations were used for the chlorine treatment; this is within the World Health Organisation regulations of less than 5mg/L chlorine concentration for drinking water disinfection.

High Density Polyethylene (HDPE) pipe surfaces were cut into sizes of approximately 13.5 cm^2, cleaned and sterilized for use to simulate the drinking water distribution pipes mostly and commonly used around the Globe. This study work was conducted in sterile environments under a laminar flow chamber.

Reasoner's 2A (R2A) growth medium was used for the growth of the biofilms which is a microbiologically used medium for the cultivation of most portable water bacteriology. Clean, sterilized, and dry pipe surfaces were sub-merged into sterile medium-size Petri dishes. Each petri dish contained 70ml of sterile R2A broth which was inoculated with 0.7ml of the original suspension of the bacteria strain and could accommodate 7 pieces of the pipe surfaces.

After one hour of inoculation under the laminar flow, the inoculated pipe surfaces were rinsed with sterile water approximately 10 ml for each pipe surface and then transferred into large-size petri dishes each containing 140 ml of sterile R2A broth. The large petri dish could accommodate 9 pipe surfaces ready for storage time under the treatment of interest.

The total used pipe surfaces for the storage time of 14 days for both strains were 42. Three from each strain were swabbed immediately after the one-hour inoculation using sterile cotton swabs. With sterile pair of scissors, the swab tips were cut into 9ml sterile diluents and vortexed. The three replicates of each strain were then pour-plated under the laminar chamber and later incubated at 30ºC for 48 hours, after which they were enumerated using a colony counter. The other sampling days followed 24 hours, 48 hours, 7 days, 10 days, and 14 days, respectively.

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

With a gradual growth at 15 ºC, this temperature was used as the control for the chlorine effects investigation. The procedure was the same as the temperature treatments except that the chlorine concentration under study was added into the sterile R2A broth, mixed well then measured procedurally.

0.1mg/l chlorine concentration had an outright more effective growth suppression effect on the biofilms than the 1mg/l chlorine concentration whereby the biofilms grew even higher than the control. However, in both strains, there was no chlorine effect observed during the one-hour inoculation. Regardless of the initial concentration of the original suspensions, an almost similar number of biofilms were recorded after inoculation.

It was also observed that there was no suppression growth effect on both strains after the 24 hours of storage time by the two chlorine treatments. 0.1mg/L which was the most effective, its effectiveness was recorded after 7 days of storage through 10 and 14 days respectively.

It is, therefore, concluded from this study that *Pseudomonas aeruginosa* biofilms in drinking water distribution systems were influenced positively by elevated temperatures and negatively by low temperatures. Chlorine effects were effective at low concentrations (0.1mg/l) however it took 48 hours for the effectiveness to be visible.

For the 1mg/L chlorine concentration, there is a need for more research in several dynamics to rule out an error, test *Pseudomonas aeruginosa* chlorine resistance, and understand if there is any relation between the initial concentration of the bacterial suspensions. The results from the first one-hour of sampling formed a major assumption that there was an even distribution of the same amount to the rest of the pipe surfaces.