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Role of biofilms in improving microbial quality in rainwater tanks

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ABSTRACT

To evaluate the role of biofilms in improving microbial quality in rainwater tanks, the cell number of *Pseudomonas aeruginosa* was investigated after inoculation in pilot and full-scale rainwater tanks with different surface-to-volume (S/V) ratios. In pilot-scale experiments, the total number of inoculated cells sampled from the tank water, bottom and walls decreased. After 4 and 5 d, 99% of the inoculated cells were removed from the water in pilot tanks 2 and 1, respectively. Cell death due to low-nutrient conditions contributed to the observed cell number decline. The high removal rate in pilot tank 2 was because of the high S/V ratio. Further, the high removal rate of planktonic *P. aeruginosa* in full-scale experiment was confirmed in the tanks with high S/V ratios. Our observations indicated that biofilms play a significant role in improving the quality of stored rainwater.

Keywords: Biofilms; Microbial quality; *P. aeruginosa*; Rainwater tank; S/V ratio

1. Introduction

Rainwater management system has advantages such as simple technology, low cost and low energy consumption; however, rainwater use is limited because of the uncertainty about the rainwater quality, especially its microbial quality [1]. Several studies in various countries have demonstrated the presence of faecal indicator bacteria and pathogens in rainwater storage tank [2–4]. Meanwhile, Evans et al. have suggested that though faecal indicator bacteria have access to the rainwater tank they may be regulated by micro ecosystems within rainwater tank [5].

Biofilms are one of the factors influencing microbial quality in rainwater tanks. They are layers of bacteria that develop rapidly on surfaces under low-nutrient conditions [6,7]. They shelter microbes from the environmental stress caused by disinfectants and nutrient limitations [8]. Many studies have indicated negative effects of the presence of a biofilm, such as biofouling of filters and biocorrosion and biocontamination in drinking water distribution networks; however, positive effects such as the use of biofilm reactors for the degradation or production of chemical substances in the wastewater treatment process have also been shown [9–11]. Moreover, it has been suggested biofilms may have positive effects on water quality in rainwater tanks [1,12].

However, to date, the role and impact of biofilms on the bacterial quality of stored rainwater have not been precisely defined. In this study, we investigated the microbial behaviour in pilot and full-scale

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rainwater tanks with different surface-to-volume (S/V) ratios to evaluate the role played by biofilms in improving the microbial quality in rainwater tanks.

2. Materials and methods

2.1. Pilot batch tanks

To investigate the behaviour of microbial populations in spike tests in pilot batch tanks with different S/V ratios, two 200 L polyethylene tanks were filled with 100 L of rainwater. The S/V ratios in the 2 tanks were set to 10/m and 50/m by placing acrylic plates (50 × 20 × 0.2 cm) (Fig. 1). To ensure the attachment of a sufficient amount of biofilm to the tank walls before the spike test, the rainwater (100 L) in the tanks was stored for four weeks. Thereafter, 10 L of rainwater was replaced every day and the retention time was controlled at 10 d. The water was stored in the dark at room temperature (20˚C).

2.2. Full-scale tanks

Two full-scale rainwater tanks that had been operating at Seoul National University for 3 years were employed to investigate the behaviour of their microbial populations in spike tests carried out with different S/V ratios (Fig. 2). Tank 1 was designed as a simple concrete square with a volume of 20 m^3. Tank 2 was assembled from polypropylene units with a 95% pore space and a storage volume of 20 m^3. The S/V ratios were 2/m and 15/m in Tank 1 and Tank 2, respectively. The retention time was 10 d. The difference in the material of the two tanks was assumed to be negligible because the material primarily affects the initial steps in biofilm formation [13] and the two rainwater tanks used had been operating for 3 years.

2.3. Bacterial preparation and inoculation

Pseudomonas aeruginosa (Korean Collection for Type Cultures 1636), a ubiquitous environmental bacterium that forms biofilms on wet surfaces such as those of rocks and soil [14], was used in the spike tests. *P. aeruginosa* was grown to the exponential phase (optical density at m [OD_{600}] = 1.2; approximately 5×10^7 colony forming units (CFU)/mL) in Luria-Bertani broth and washed twice with phosphate-buffered saline (centrifugation at 8,000 rpm for 10 min at 4˚C). *P. aeruginosa* was inoculated into the tanks at a final concentration of approximately 5×10^5 CFU/mL for the pilot tests and 1.3×10^4 CFU/mL for the full-scale tests. In the pilot tests, after inoculation, the *P. aeruginosa* cells were dispersed by stirring at 180 rpm for 10 min. The pilot test was performed in batches.

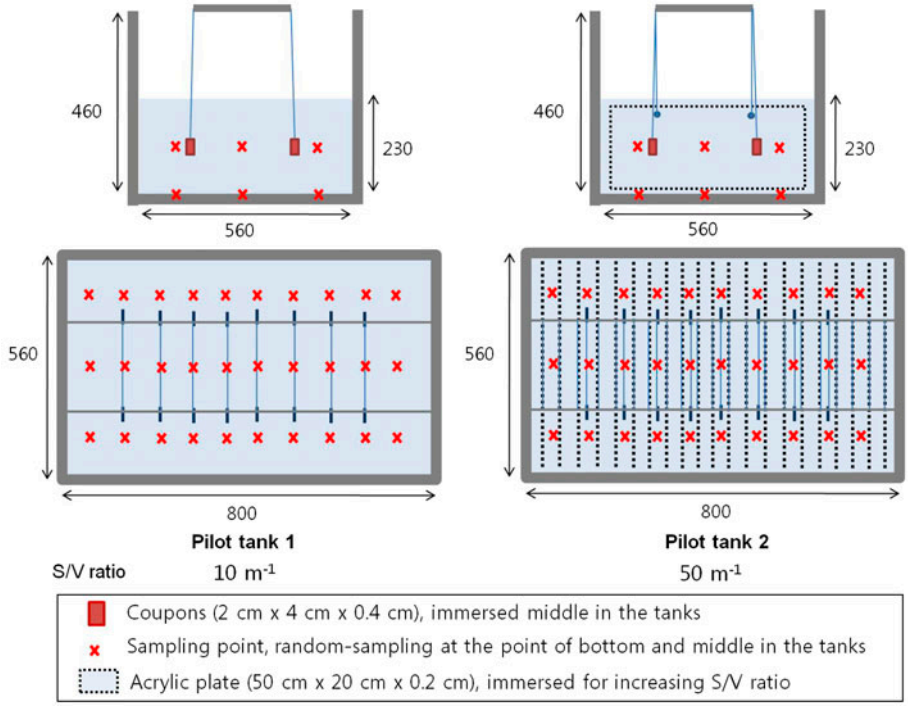

Fig. 1. Schematic diagram for pilot-scale batch experiments.

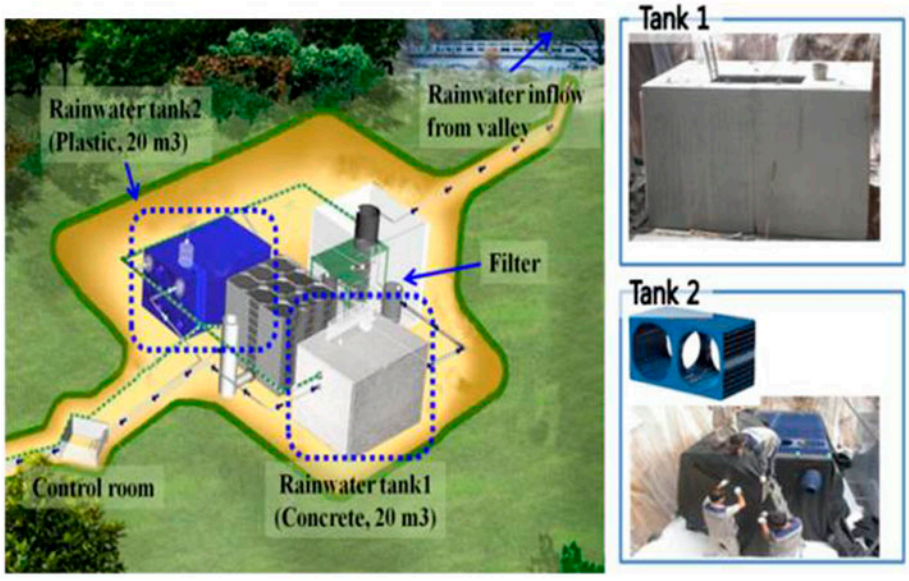

Fig. 2. Schematic diagram and photographs of rainwater tanks used in full-scale experiments.

2.4. Sampling and bacterial counts

To investigate the behaviour of *P. aeruginosa* in the water and on the bottom and walls of the tanks, 50 mL water samples were taken in duplicate from the bottom and middle sections of the two tanks in the pilot test, and coupons were tested randomly every day for 8 d (Fig. 1). In the full-scale test, 1 L water samples were taken in duplicate from the two tanks every day for 10 d to determine the *P. aeruginosa* concentration in the rainwater. To quantify the bacteria by using the conventional microbiological culture method, an aliquot of each sample was serially diluted and spread in duplicate on MacConkey agar plates (Difco; Becton, Dickinson & Co., USA) selective for faecal coliforms. As a control measure, it was confirmed that no colony of *P. aeruginosa* was present in the water samples taken from the tanks, prior to inoculation. The colonies found after inoculation were thus regarded and counted as those of *P. aeruginosa*. After incubation at 35˚C for 24 h, we counted the CFU for each plate, calculated the average and expressed the results as CFU/mL. The pH value in both the pilot tanks was 7.1 ± 0.1 and dissolved oxygen was 7.9 ± 0.5 and 6.7 ± 0.4 mg/L in pilot tanks 1 and 2, respectively.

2.5. Data analysis

The removal rates of *P. aeruginosa* in the rainwater were determined by calculating the slope. Correlation coefficient (r^2) of the linear regression of log-transformed cell concentration data according to the first-order decay equation, given as $C = C_0 e^{-kt}$, where C is the microbial concentration at time t, C_0 is the initial concentration on day 0 and k is the removal rate.

3. Results and discussion

3.1. Removal of P. aeruginosa from rainwater

The persistence of the *P. aeruginosa* cells inoculated into the pilot tanks was because of the interaction between cell growth and death rates and that between the attachment, detachment and sedimentation processes. The total number of inoculated cells in samples taken from water, bottom and walls inside the tanks decreased in both the tanks (Fig. 3). Cell death contributed more to the observed cell number decline than did cell growth under the experimental conditions.

Fig. 4 shows the removal rate of *P. aeruginosa* from the water in the two pilot tanks. Ninety-nine percent of the inoculated *P. aeruginosa* cells were removed from pilot tanks 1 and 2, after 5 and 4 d, respectively. The high removal rate in pilot tank 2 was because of the high S/V ratio.

3.2. Behaviour of P. aeruginosa inoculated into pilot tanks

During the experiment, the number of *P. aeruginosa* cells in the water declined by 3–4 log units, indicating that the death, attachment and sedimentation processes dominated the overall bacterial dynamics (Fig. 5(A)). At the same time, the removal rate of *P. aeruginosa* in the water phase was –0.57 $\log_{10}$ cells/mL/d (r^2 = 0.93) in pilot tank 1 and –0.74 $\log_{10}$ cells/mL/d (r^2 = 0.98) in pilot tank 2. A high removal rate was seen in tank 2, which had a high S/V ratio.

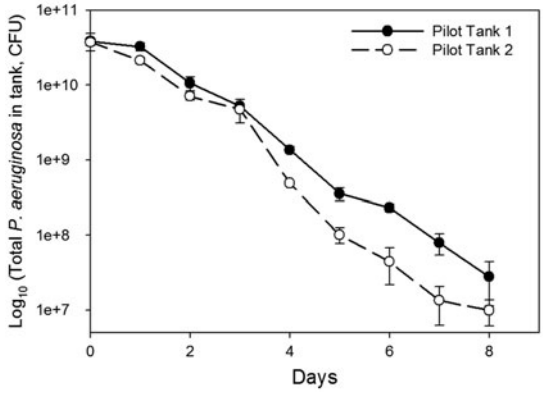

Fig. 3. Total amount of *P. aeruginosa* inoculated in pilot tanks.

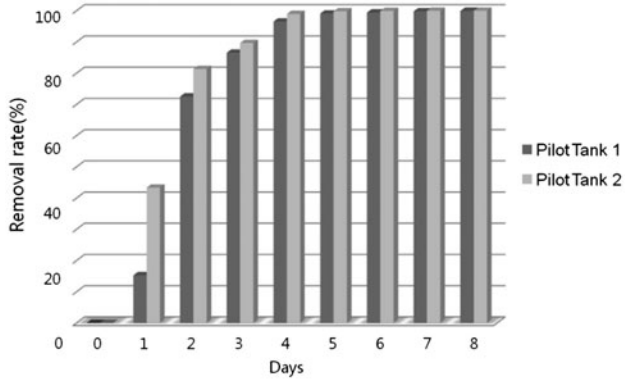

Fig. 4. Removal rate of *P. aeruginosa* in pilot tanks.

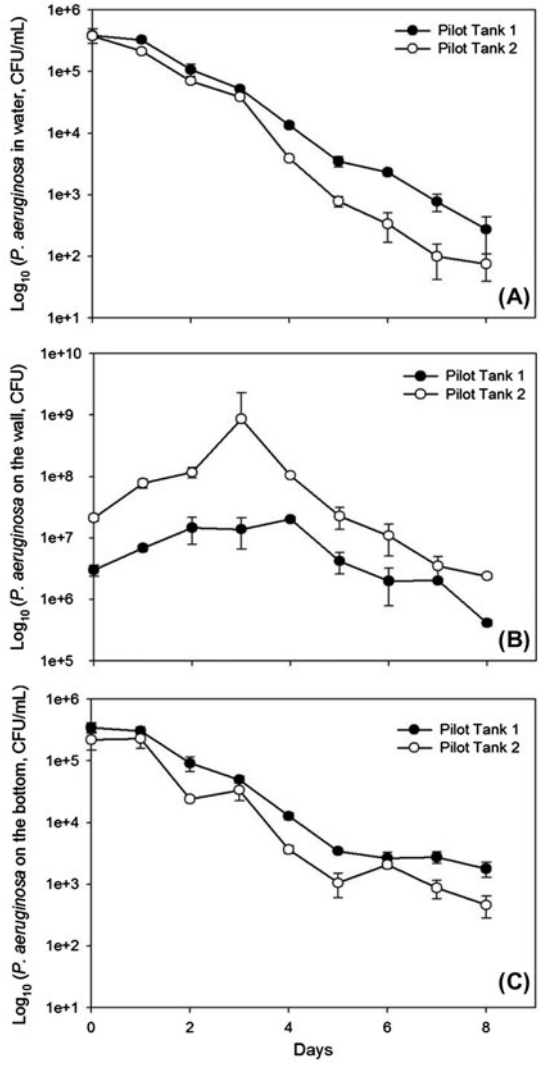

Fig. 5. Behaviour of *P. aeruginosa* (A) in the water, (B) on the walls and (C) at the bottom of pilot tanks.

The number of attached *P. aeruginosa* cells increased for 4 d in pilot tank 1 and for 3 d in pilot tank 2 (Fig. 5(B)). Their attachment to the biofilm on the walls initially dominated the bacterial dynamics and more bacteria were attached in tank 2 than in tank 1 because of the high S/V ratio of the former.

After 4 d, the number of attached *P. aeruginosa* cells declined by 2–3 log units, indicating that the death and detachment processes dominated the bacterial dynamics on the walls (Fig. 5(B)). Established biofilms developed from indigenous river water bacteria have been shown to reduce the persistence of introduced *Escherichia coli* and other enteric pathogens [15]. Banning et al. showed that under certain conditions, the presence of mixed-population biofilms may limit the survival potential of enteric bacterial pathogens introduced into groundwater [16]. In addition, changes in biofilm dynamics and pathogen persistence are influenced by increase in nutrient levels. Buswell et al. showed a significant decline in the survival rate of the *Campylobacter jejuni* strain in heterogeneous tap water biofilms, following the addition of serine, a carbon source favoured by *C. jejuni* and a concurrent increase in the number of indigenous biofilm microflora [17,18]. These studies demonstrate that under certain conditions, biofilms represent the sites of intensified competition for limited nutrients. Thus, in the present study, the decrease in the number of *P. aeruginosa* cells in the biofilms in the oligotrophic rainwater tanks might have resulted from the competition for nutrients with indigenous microbial communities. The inoculated *P. aeruginosa* cells were found at the bottom of the tanks and they decayed over time (Fig. 5(C)). More bacteria settled at the bottom of tank

1, which had a low S/V ratio, while more bacteria attached to the biofilm on the walls in tank 2.

3.3. Behaviour of P. aeruginosa inoculated into full-scale tanks

During this experiment, the number of *P. aeruginosa* cells in the water declined by 1.5 log units in tank 1 and by 2 log units in tank 2 (Fig. 6). The removal rate was –0.604 $\log_{10}$ cells/mL/d (r^2 = 0.99) in tank 1 and –0.854 $\log_{10}$ cells/mL/d (r^2 = 0.99) in tank 2. Similar to the results of the pilot test, a high removal rate was seen in tank 2 because of its high S/V ratio. It could thus be concluded that increasing the S/V ratio in rainwater tanks to a certain level is an effective way to remove bacteria from rainwater; however, this requires the identification of the optimum S/V ratio.

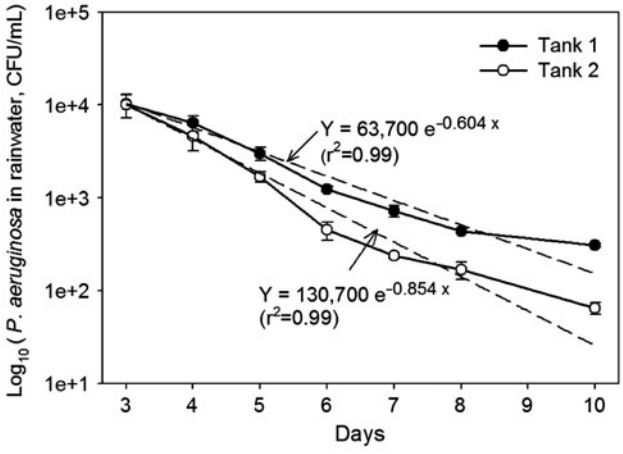

Fig. 6. Number of *P. aeruginosa* cells inoculated in full-scale tanks.

3.4. Role of biofilms in rainwater tanks

It has been suggested that rainwater tanks are unique ecosystems that support functional ecosystems comprising complex communities of environmental bacteria [5,19]. In this study, a large surface area for biofilm formation led to a high removal rate of *P. aeruginosa* in rainwater. Hence, when introduced into rainwater tanks with limited nutrient conditions, opportunistic pathogens such as *P. aeruginosa* seem to be removed because of their attachment to biofilms and die both naturally and because of competition for nutrients with indigenous microbial communities.

Fig. 7 shows the viable-cell ratios of *P. aeruginosa* inoculated in the pilot tanks. The total number of cells decreased over time and a high removal rate was observed in tank 2, which had a high S/V ratio (Fig. 3). In tank 1, *P. aeruginosa* was found in the water phase, for the most part. The viable-cell ratio on the walls increased by 2–3% at days 7 and 8, while that of <1% was observed at the bottom during the experimental period. In tank 2, the viable-cell ratio in the water was decreased after day 2 and was maintained at 75% after day 6. Other viable cells were found on the walls and bottom of tank 2. The viable-cell ratio on the walls was maintained around 25% after day 6, while that of <1% was observed at the bottom. These results showed that equilibrium is achieved in rainwater tanks over time, for which there are two possible reasons. Either the micro-organisms die off at a rate determined by the nutrients present or quorum sensing occurs, that is, these micro-organisms move to the biofilm to obtain nutrients and shelter. In *P. aeruginosa*, well known as a biofilm former, the intercellular signalling molecules are compounds. As these molecules accumulate, they signal adjacent *P. aeruginosa* cells (a mechanism called quorum sensing) and then the biofilm develops [20].

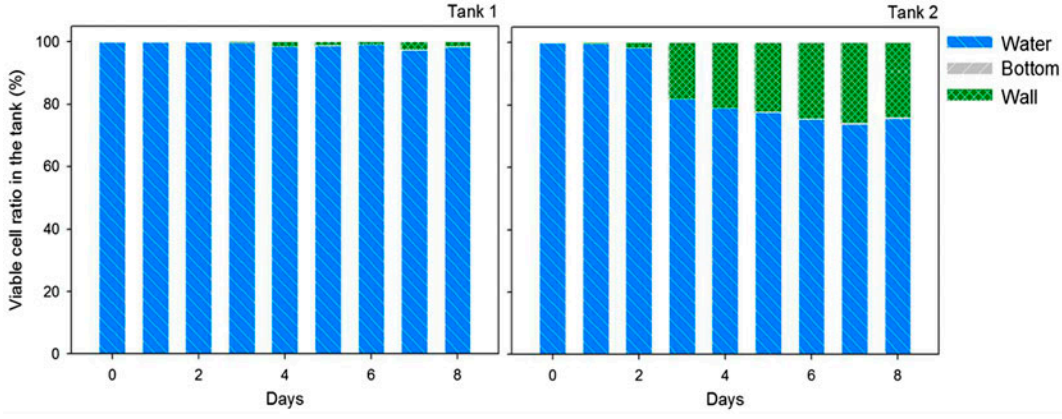

Fig. 7. Viable-cell ratio of *P. aeruginosa* in pilot tanks.

4. Conclusions

Our microbial observations in the pilot and full-scale rainwater tanks led us to the following conclusions:

(1) Pathogens, including opportunistic pathogens such as *P. aeruginosa* that are introduced into rainwater tanks under limited nutrient conditions are removed through attachment to biofilms and die both naturally and because of competition for nutrients with indigenous microbial communities. Thus, biofilms play a significant role in improving the quality of stored rainwater and they should therefore be considered in the design and operation of rainwater tanks.
(2) Increasing the S/V ratio in rainwater tanks by a certain level is an effective way of removing bacteria from the stored rainwater, improving its microbial quality.
(3) However, the appropriate range of S/V ratios for tank design could not be suggested. Therefore, further research aimed at identifying the range of S/V ratios that is most effective in improving microbial quality is proposed. The resulting information would help in the development of appropriate guidelines for the design of rainwater tanks.

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