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An analysis on biofilms in drinking water distribution system

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Abstract- An accumulation of organic and inorganic live and dead organisms, both on and off the surface, is known as a biofilm. However, little patches on pipe surfaces are more common in water systems. It might be a complete coating. Biofilms in drinking water distribution systems and pipe networks can cause a variety of operational and water quality problems. Red or black water issues caused by bacteria that break down iron or sulphate can be attributed to biofilms, along with an increase in bacterial populations, a decrease in dissolved oxygen, taste and odour alterations, and problems with taste and smell, microbially influenced corrosion, hydraulic irregularity, and decreased material life can all be attributed to biofilms.

Index Terms- bacteria, pipe networks, water distribution systems, biofilm

I. Introduction

Bacteria (including coccoid spherical, rod-shaped, filamentous, and appendaged bacteria), fungus, and higher organisms including worms, larvae, and Crustacea are just a few examples of the microorganisms that can be found in biofilms. Recent studies have demonstrated that parasites and viruses like Cryptosporidium can become enmeshed in biofilms. In spite of the fact that they cannot grow there, viruses and cryptosporidium can attach to biofilms after a contamination event. As a result, to totally rid the distribution system of these organisms after a contamination event, a flushing procedure must be performed.

Both customers and drinking water producers are very concerned about the decline in water quality brought on by bacterial growth in the supply networks. In addition to being on the surface of the pipe walls as a biofilm, bacteria, yeasts, fungi, and protozoa may also be present in the water phase. A resilient ecosystem that is difficult to eradicate is formed by these thin layers (aside from deposits or tubercules). A web of exopolymers consisting of proteins and polysaccharides is used to tightly bind microorganisms that require support and protection (LeChevallier, M. W., C. D. Lowry and R. G. Lee. 1990). Conclusions and calculations based on Camper's (1996) findings demonstrate that even in the absence of chlorine, bacterial growth in the liquid phase is negligible in a system for distributing potable water. As a result of shear loss, only the bacteria in the biofilm attached to the walls of the distribution pipework are growing, and this is one of the main causes of the deterioration in the microbiological quality of water distribution systems (LeChevallier, M. W., N. J. Welch, and D. B. Smith. 1996). Biofilms can cause a variety of problems in drinking water distribution systems, including:

- A trophic food chain can begin with bacteria and progress to higher organisms that are not desired.
- High levels of heterotrophic bacteria (HPC) prevent the identification of coliforms or other sanitary indicators. Certain bacterial species can cause turbidity, taste, and odours in drinking water.
- Biocorrosion is accelerated by the accumulation of connected biomass.
- Biofilms increase frictional resistance, which lowers the water-carrying capacity of distribution systems.
- The distribution system's ongoing inability to meet all set water quality standards.

Many water utilities start to worry about biofilms in drinking water systems due to the growth of coliform bacteria in the pipe network. In the United States alone, over 4,400 water systems affecting 21 million people in 1993 violated drinking water standards for total coliform bacteria (Camper, A. 1996). Similar patterns indicate that in 1994 and 1995, over 12,000 systems exceeded the allowable coliform levels. The nearly 2,000 systems that frequently detect coliform bacteria in finished drinking water and are flagged as seriously noncompliant raise concerns. While some of these systems occasionally experience coliform outbreaks due to cross connections and other operational concerns, a sizable portion of these systems can blame the regrowth of the bacteria in distribution system biofilms for their problems.

II. Literature review

Water quality and public health are safeguarded by drinking water distribution arrangement, which are designed and run so that drinking water's biostability is preserved from treatment to the tap. It is commonly accepted that the ability of a material its physical and chemical integrity after implantation into a living tissue. relates to preserving adequate microbiological water (Pontius, F. 1995). reducing microbial (re)growth and contamination from planktonic sources associated with distribution, Usually, the finished water is infused with a disinfectant residue. However, because the DWDS infrastructure actually houses the majority of the microbial biomass, biofilms—mixed microbial taxonomic communities embedded in extracellular polymeric substances—develop on the interior surfaces of the DWDS infrastructur (Prest, E. I., Hammes, F. *et al*., 2016) . More people are becoming aware of the potential for biofilms to impair the quality of water through they mediate processes through, for example, moving from the pipe wall into the water column. The public's health could be put at risk if germs are unleashed, which could result in aesthetic failures (Fish *et al*., 2016). Despite evidence showing that the microbiota in biofilms and the planktonic microbiome are distinct (Roeselers *et al.*, 2015), The standard of planktonic microbial communities is primarily taken into account in regulations governing water quality and disinfection practises. Since it is inexpensive, simple to use, and has a wide range of activation, chlorine is an often used disinfection residue (Donnermair and Blatchley, 2003) Despite the fact that disinfected drinking waters commonly have concentrations between 0.2 and 1.0 mgL-1, the World Health Organisation [WHO] (2003) now advises using biocide residuals (including chlorine) at no more than 5 mgL-1. But in addition to concentration, other factors such as pH, contact time, hydrodynamics, temperature, and chlorine demand also affect chlorine efficacy, Our present knowledge of chlorination is mostly focused on how it affects planktonic cells and, operationally, applications of disinfection in the context of water treatment that is centred on bulk water. The aforementioned parameters don't apply to the DWDS because it has a high surface-area-to-volume ratio (Fish *et al*., 2016).

Despite the usage of disinfection residuals, bacteria, fungi, archaea, viruses, and amoeba have established a diverse population in the DWDS due to the persistence of microorganisms in treated water (Potgieter *et al*., 2018). These bacteria colonise the internal surfaces of DWDS or adhere to preexisting biofilms even if they are inactivated or damaged. The existence of residual chlorine has no influence on the establishment of biofilms and may instead encourage their growth because bacteria in a model DWDS that had its chlorine content increased favoured the biofilm state (Su *et al.* 2018).

It's been established that, across a range of settings, Compared to their planktonic counterparts, Bacteria and fungi that are attached to biofilms are more resilient to residuals and may sustain stronger disinfectant dosages (Hageskal *et al*., 2012).

There is broad consensus that EPS act as a barrier to give physical protection., albeit the precise mechanisms underlying this are still up for debate (Xue *et al*., 2013). As a result of biofilms' resistant for sanitization, DWDS must apply more chlorine, which can have an adverse effect on the water's aesthetics (taste and odour) and raise operational expenses.

It is currently unknown how residual chlorine affects the common (but sometimes disregarded) systems with large-scale DWDS biofilm microbiomes. Knowing how a network of sources and sinks might affect how chlorine affects biofilms the residue degrades and the concentration varies is especially important. Prior studies largely examined how disinfectants affected the ecology of planktonic microorganisms (Potgieter *et al*., 2018).

Although the hydraulics and scale of these systems are not comparable to real DWDS, a few studies analysing biofilms in simulated distribution systems found that the disinfectant utilised (chlorine or chloramines) had an effect on the microbiota. The effects and interactions between chlorine and biofilms produced employing certain, preselected drinking water bacteria have also been emphasised by bench-top experiments (Gomes *et al*., 2016; Lin *et al*., 2017). The results of these investigations suggest that changing how items are disinfected affects the makeup of microbial communities and the amount of bacteria present by either favouring (or disfavoring) particular bacterial groups or by changing the proportion of bacterial or eukaryotic taxa present without altering the existing taxonomic species (Lin *et al*., 2017, Wang *et al*., 2014).

Using chlorine residuals to minimise planktonic bacteria or cleaning pipes mechanically with Only two operational practises that have an indirect impact on modern biofilm control techniques are high flow rates and clearing debris from pipe walls (Husband and Boxall, 2011). Since these methods weren't developed specifically for the management of biofilm formation and persistence, there are no criteria or guidelines for how frequently such interventions should be done. Investigating how "cleaning" affects DWDS, In order to comprehend the dynamics of the biofilm and chlorine in this system, biofilms is a critical next step This study's primary objective was to determine how the DWDS biofilm microbiota was impacted by chlorine concentration, in particular, the effects of chlorine on the variety of taxa, the composition of communities, and the populations of bacteria and fungus. A secondary objective was to compare the regeneration of the bacterial and fungal communities after mechanical cleaning (again, at various chlorine concentrations) to the initial microbiome development.

III. Factors Affecting Coliform Occurrences

To find out what causes coliform bacteria to show up in drinking water, recent research have looked at information from approximately 80 water systems (LeChevallier *et al*., 1996; Volk *et al*., 1996). These investigations have demonstrated that a variety of factors, including filtration, temperature, the kind and residue of the disinfectant, the amount of assimilable organic carbon (AOC), the efficiency of corrosion control, and the choice of pipe material, might affect the presence of coliform bacteria.

- **Filtration**

Four unfiltered surface water systems were used in one study. While this only accounted for 26.7 percent of the total number of bacterial samples collected, it produced 64.4 percent (1,014 out of 1,577) of the positive coliform sample results. The data did not indicate that the treatment was ineffective (coliforms, for example, were not associated with overcoming treatment barriers), but they did suggest that filtration may be necessary for preventing coliform recurrence (LeChevallier *et al.,* 1996)). The trial led to a three-fold reduction in coliform levels during the subsequent 17 months in one of the installed filtration and distribution systems.

- **Temperature**

Coliform bacteria were significantly more prevalent in water that was over 15°C. However, there were differences between systems in the lowest temperature at which microbial activity was found. During certain times, the water's close to 10°C, coliform incidences increased in systems that generally dealt with cold water. These systems' coliform bacterial strains may be more suited to growing at low temperatures (psychrophiles).

- **Disinfectant Level and Residual Disinfectant**

There is a difference between filtered systems that employ chloramines and those that maintain a residual of free chlorine. While 0.97 percent of 33,196 samples from systems utilising free chlorine did not include coliform bacteria, 0.52 percent of 35,159 samples from chlorinated systems did (statistically different at p.0001) The average density of coliform bacteria was found to be 36 times higher than free chlorinated systems, despite the fact that chlorinated water has a lower colony-forming unit (CFU) density (0.60 CFUs/100 ml against 0.017 CFUs/100 ml, respectively). According to earlier studies (LeChevallier *et al.,* 1990; LeChevallier, 1991), In distribution systems, chloramines would more effectively pierce biofilms and eradicate bacterial adhesion. Because different disinfectants have various methods of action, they may interact with biofilms in different ways. Trihalomethanes are known to arise when free chlorine reacts with naturally occurring organic materials (Rook, 1974). These products are not formed by chloramines to the same extent. Gram-negative bacteria are subjected to free chlorine assault, which induces cellular damage, cellular lesion, and increased susceptibility to surfactants (Zaske *et al.,* 1980).

A reducing agent (sodium sulfite) can treat the lesion that chloramines generated since they do not damage materials in the same manner that free chlorine does (Watters *et al*., 1989). It has been modelled and demonstrated that free chlorine's quick response rate restricts the amount of free chlorine that can enter a biofilm (LeChevallier, 1991; DeBeer *et al*., 1994).

Before it interacts with the bacterial elements of the film, free chlorine is essentially removed. Chloramines, on the other hand, take longer to react and can seep into the biofilm, where they ultimately inactivate the clinging bacteria. Researchers at Montana State University expertly represented this mechanism using an alginate bead model (Chen and Stewart, 1996). Free chlorine could not successfully permeate alginate beads harbouring bacterial cells, but chloramines might, according to research by Stewart and colleagues (in press) (2.5 mg/L, pH 8.9) reduced bacterial counts by roughly a million times over the course of 60 minutes.

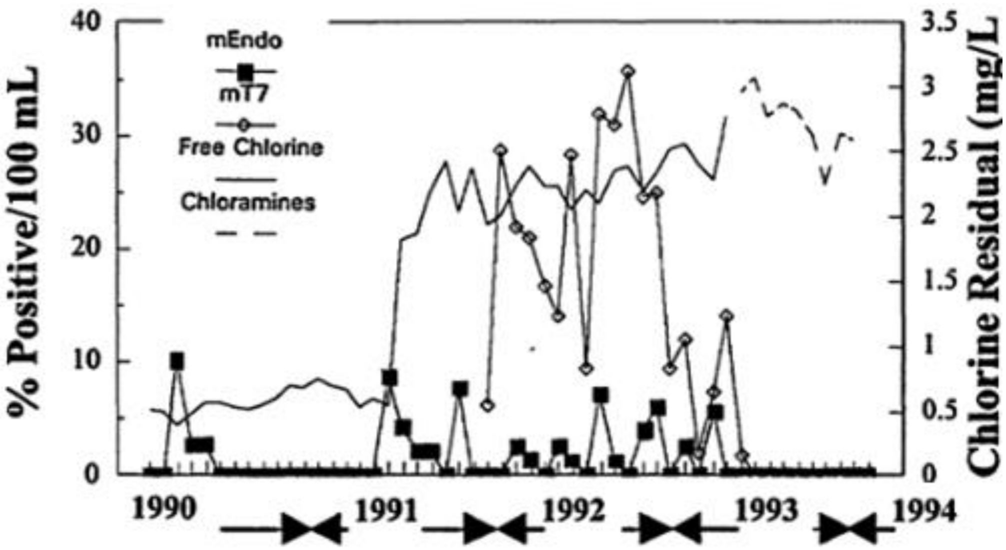

Figure 1: Pre- and post-chlorine conversion coliform incidence in a system.

Figure no.1 shows how effective a chloramine residual is at preventing coliform outbreaks, which are assumed to be caused by the growth of biofilm in distribution networks. Coliform occurrences continued to occur despite distribution system free chlorine residuals often between 2 and 2.5 mg/L on average. When utilising the technique of recovering wounded bacteria known as m-T7 media, coliform incidence rates ranged from 10 to 40% (LeChevallier *et al.,* 1983). This was true even in the months when coliforms were not found on the typical m-Endo medium. When the disinfectant was changed to chloramines in June 1993, measurements of coliform incidences in both the m-Endo and m-T7 media drastically dropped, and three years after the changeover, the germs have not been discovered in completed drinking water (Norton and LeChevallier, 1997).

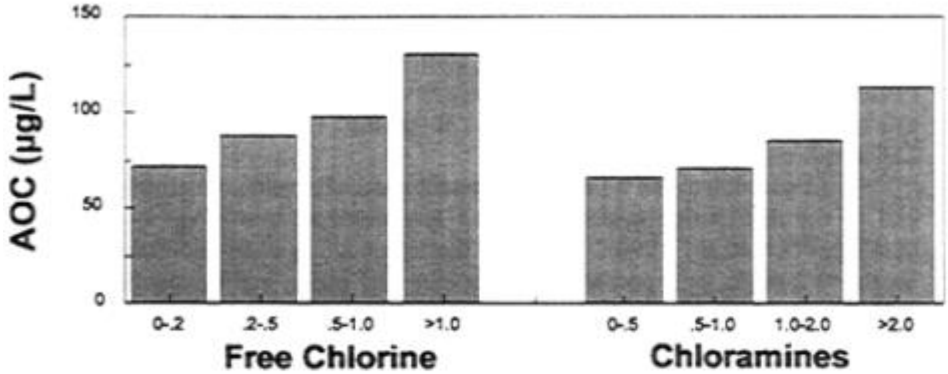

Figure 2: Relationship in the distribution system between AOC and disinfectant residuals.

In addition to the disinfectant used, the residue kept at the end of the distribution system was connected to the occurrence of coliform (LeChevallier *et al.*, 1996) Comparatively to systems with higher dead-end free chlorine or monochloramine levels of less than 0.5 mg/L, systems with smaller disinfection residuals exhibited significantly higher coliform incidences. However, to maintain high disinfection residuals and prevent coliform outbreaks, high assimilable organic carbon (AOC) systems were required. As a result, treating waters alone did not guarantee that coliform bacteria would not be present.

IV. The Importance of Biofilm Control for Public Health

If coliform bacteria growth in distribution system biofilms had no impact on public health, it might be seen as an annoyance. Coliform bacteria have historically been used to assess how well drinking water has been treated. The treatment of drinking water is insufficient, according to a novel interpretation of this indicator principle, if coliform bacteria can grow on biofilms prevalent in distribution systems. Concerning opportunistic illnesses like Legionella pneunophila, Mycobacterium avium complex (MAC), or other microorganisms that can reproduce is one thing.

The presence of Mycobacterium intracellulare and M. avium complex members in drinking water distribution systems has been shown to range from 0.08 to 4, 000 CFUs/ml. Between 25 and 50 percent of AIDS patients develop lethal and debilitating M. avium complex infections, which have been found to account for the bulk of these illnesses (Horsburgh, 1991; Nightingale *et al.*, 1992). The reality that the individual affects the lungs or digestive system raises the possibility that AIDS patients may contract the disease through drinking water or eating certain foods.

Using a chloramine residual, An ongoing research study that looked at eight well-known drinking water systems and collected samples from the raw water and distribution systems frequently discovered slow-growing mycobacteria. Using either free-chlorine or ozone treatment, mycobacteria in plant effluent levels seemed to be adequately eradicated to be below detectable levels. Considering that unfiltered water often contains mycobacteria and because they might be removed if they grow too big for the selective medium, it might be too soon to say that chloramines provide mycobacteria a selection advantage. It is unclear why there are so many slow-growing mycobacteria at the groundwater location with free chlorine, but this could be because of the region's low chlorine residuals, which only average 0.15 mg/L.

The most widely used preventive measure to stop the development of biofilm is disinfection, which renders any existing planktonic microbes inactive. Chlorination (Cl2) is widely used as chlorine; it is an affordable chemical with persistent and broad biocidal effects such as damage to DNA, proteins, lipids, and other cell components (Douterelo, I.; Calero-Preciado, *et al.*, 2018) . However, chlorine has a limited impact on bacteria that are resistant to it and can produce toxic by- products (Zheng, J.; Su, C.; Zhou, *et al*., 2017). Another relatively inexpensive method of disinfecting drinking water is ultraviolet (UV), which produces no byproducts (Li, X.; Cai, M.; Wang, L.; Niu,*et al*., 2019, and Choi, Y.-J.2010) . LEDs are lights that emit light. UV is a competitive source of light for UV disinfection because of its safety, high efficacy, tailored wavelength combination, and small size (Luo, X.; *et al*., 2022 and Torkzadeh, H.; *et al*., 2021) . However, UV radiation lacks durable inactivation because it primarily kills bacteria by dimerizing pyrimidine, which can be repaired (Ghosh, S.; *et al.*, 2022).

V. Conclusion

Whether coliform renewal takes place in distribution systems is impacted by complex interactions between chemical, physical, operational, and engineering factors. The water utility operator must take into account all of the aforementioned factors in order to come up with a solution to the regrowth problem because no single cause could possibly explain for all instances of coliform. To reduce the prevalence of opportunistic diseases like Mycobacterium avium complex in sources of drinking water, even coliform-free systems may want to pay closer attention to biofilm management strategies and procedures.

References

[1] 1. LeChevallier, M. W., C. D. Lowry and R. G. Lee. 1990. Disinfecting biofilms in a model distribution system. Journal of the American Water Works Association 82(7):87.

[2] 2. LeChevallier, M. W., N. J. Welch, and D. B. Smith. 1996. Full scale studies of factors related to coliform regrowth in drinking water. Applied Environmental Microbiology 62:2201.

[3] 3. Camper, A. 1996. Factors Limiting Microbial Growth in Distribution Systems: Laboratory and Pilot-Scale Experiments. Denver, Colo.: American Water Works Association Research Foundation, ISBN: 978-08-98678-76-5.

[4] 4. Pontius, F. 1995. SDWA Advisor on CD. Denver, Colo.: American Water Works Association.

[5] 5. Prest, E. I., Hammes, F., van Loosdrecht, M. C. M., and Vrouwenvelder, J. S. (2016). Biological stability of drinking water: controlling factors, methods, and challenges. *Front. Microbiol.* 7:45. doi: 10.3389/fmicb.2016.00045.

[6] 6. Flemming, H., Percival, S., and Walker, J. (2002). Contamination potential of biofilms in water distribution systems. Water Sci. Technol. 2, 271–280. doi: 10.2166/ws.2002.0032

[7] 7. Fish, K. E., Husband, S. P., and Boxall, J. B. (2016). "The impact of chlorine concentration on the discolouration response of biofilms in drinking water distribution systems," in Proceedings of the WDSA-CCWI 2018, Kingston, ON.

[8] 8. Roeselers, G., Coolen, J., van der Wielen, P. W. J. J., Jaspers, M. C., Atsma, A., de Graaf, B., et al. (2015). Microbial biogeography of drinking water: patterns in phylogenetic diversity across space and time. Environ. Microbiol. 17, 2505–2514. doi: 10.1111/1462-2920.12739.

[9] 9. Donnermair, M. M., and Blatchley, E. R. (2003). Disinfection efficacy of organic chloramines. Water Res. 37, 1557–1570. doi: 10.1016/S0043-1354(02)00522-5.

[10] 10. World Health Organisation [WHO] (2003). WHO Chlorine in Drinking-Water. Available at: http://www.who.int/water_sanitation_health/publications/chlorine/en.

[11] 11. Fish, K. E., Husband, S. P., and Boxall, J. B. (2016). "The impact of chlorine concentration on the discolouration response of biofilms in drinking water distribution systems," in Proceedings of the WDSA-CCWI 2018, Kingston, ON.

[12] 12. Potgieter, S., Pinto, A., Sigudu, M., du Preez, H., Ncube, E., and Venter, S. (2018). Long-term spatial and temporal microbial community dynamics in a large-scale drinking water distribution system with multiple disinfectant regimes. Water Res. 139, 406–419. doi: 10.1016/j.watres.2018.03.077.

[13] 13. Su, H.-C., Liu, Y.-S., Pan, C.-G., Chen, J., He, L.-Y., and Ying, G.-G. (2018). Persistence of antibiotic resistance genes and bacterial community changes in drinking water treatment system: from drinking water source to tap water. Sci. Total Environ. 61, 453–461. doi: 10.1016/j.scitotenv.2017.10.318.

[14] 14. Hageskal, G., Tryland, I., Liltved, H., and Skaar, I. (2012). No simple solution to waterborne fungi: various responses to water disinfection methods. Water Sci. Technol. 12:220. doi: 10.2166/ws.2012.131.

[15] 15. Xue, Z., Hessler, C. M., Panmanee, W., Hassett, D. J., and Seo, Y. (2013). Pseudomonas aeruginosa inactivation mechanism is affected by capsular extracellular polymeric substances reactivity with chlorine and monochloramine. FEMS Microbiol. Ecol. 83, 101–111. doi: 10.1111/j.1574-6941.2012.01453.x

[16] 16. Potgieter, S., Pinto, A., Sigudu, M., du Preez, H., Ncube, E., and Venter, S. (2018). Long-term spatial and temporal microbial community dynamics in a large-scale drinking water distribution system with multiple disinfectant regimes. Water Res. 139, 406–419. doi: 10.1016/j.watres.2018.03.077.

[17] 17. Gomes, I. B., Simoes, M., and Simoes, L. C. (2016). The effects of sodium hypochlorite against selected drinking water-isolated bacteria in planktonic and sessile states-science direct. Sci. Total Environ. 565, 40–48. doi:10.1016/j.scitotenv.2016.04.136.

[18] 18. Lin, H., Xuan, Z., Yuxin, W., and Xin, Y. (2017). Effect of sodium hypochlorite on typical biofilms formed in drinking water distribution systems. J. Water Health 15, 218–227. doi: 10.2166/wh.2017.141.

[19] 19. Husband, P. S., and Boxall, J. B. (2011). Asset deterioration and discolouration in water distribution systems. Water Res. 45, 113–124. doi: 10.1016/j.watres.2010.08.021.

[20] 20. LeChevallier, M. W., N. J. Welch, and D. B. Smith. 1996. Full scale studies of factors related to coliform regrowth in drinking water. Applied Environmental Microbiology 62:2201.

[21] 21. LeChevallier, M. W., C. D. Lowry and R. G. Lee. 1990. Disinfecting biofilms in a model distribution system. Journal of the American Water Works Association 82(7):87.

[22] 22. Zaske, S. K., W. S. Dockins, and G. A. McFeters. 1980. Cell envelope damage in E. coli caused by short term stress in water. Applied Environmental Microbiology 40(2):386-390.

[23] 23. Watters, S. K., B. H. Pyle, M. W. LeChevallier, and G. A. McFeters. 1989. Enumeration of Enterobacter cloacae after chloramine exposure. Applied Environmental Microbiology 55:322.

[24] 24. De Beer, D., R. Srinivasan, and P. S. Stewart. 1994. Direct measurement of chlorine penetration into biofilms during disinfection. Applied Environmental Microbiology 60:4339.

[25] 25. Chen, X., and P. S. Stewart. 1996. Chlorine penetration into artificial biofilm is limited by a reaction-diffusion interaction. Environmental Science and Technology 30:2078.

[26] 26. Norton, C. D., and M. W. LeChevallier. 1997. Chloramination: Its effect on distribution system water quality. Journal of the American Water Works Association. 89(7):66-77.

[27] 27. Douterelo, I.; Calero-Preciado, C.; Soria-Carrasco, V.; Boxall, J.B. Whole Metagenome Sequencing of Chlorinated Drinking Water Distribution Systems. *Environ. Sci. Water Res. Technol.* 2018, *4*, 2080–2091.

[28] 28. Zheng, J.; Su, C.; Zhou, J.; Xu, L.; Qian, Y.; Chen, H. Effects and Mechanisms of Ultraviolet, Chlorination, and Ozone Disinfection on Antibiotic Resistance Genes in Secondary Effluents of Municipal Wastewater Treatment Plants. *Chem. Eng. J.* 2017, *317*, 309–316.

[29] 29. Li, X.; Cai, M.; Wang, L.; Niu, F.; Yang, D.; Zhang, G. Evaluation Survey of Microbial Disinfection Methods in UV-LED Water Treatment Systems. *Sci. Total Environ.* 2019, *659*, 1415–1427.

[30] 30. Choi, Y.; Choi, Y.-J. The Effects of UV Disinfection on Drinking Water Quality in Distribution Systems. *Water Res.* 2010, *44*, 115–122.

[31] 31. Luo, X.; Zhang, B.; Lu, Y.; Mei, Y.; Shen, L. Advances in Application of Ultraviolet Irradiation for Biofilm Control in Water and Wastewater Infrastructure. *J. Hazard. Mater.* 2022, *421*, 126682.

[32] 32. Torkzadeh, H.; Cates, E.L. Biofilm Growth under Continuous UVC Irradiation: Quantitative Effects of Growth Conditions and Growth Time on Intensity Response Parameters. *Water Res.* 2021, *206*, 117747.

[33] 33. Ghosh, S.; Chen, Y.; Hu, J. Application of UVC and UVC Based Advanced Disinfection Technologies for the Inactivation of Antibiotic Resistance Genes and Elimination of Horizontal Gene Transfer Activities: Opportunities and Challenges. *Chem. Eng. J.* 2022, *450*, 138234.

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