Ultraviolet dose screening to minimize bacterial regrowth in a bench-scale study

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Highlights:

- Increasing the UV radiation dose has been shown to contribute to minimizing post disinfection regrowth.
- Dark repair of *Escherichia coli* was observed at all UV radiation tested doses, especially at 10 mJ/cm² and 20 mJ/cm².
- UV radiation dose of 50 mJ/cm² under indirect sunlight kept *Escherichia coli* latent for 48 h.

Keywords: Disinfection; *Escherichia coli*; Inactivation.

INTRODUCTION

Groundwater is one of the primary sources of supply for populations living in low-income regions and rural communities (Carrard et al., 2019), and in many cases, due to its quality, a simple disinfection step is recommended to make it safe for human consumption. Consequently, ultraviolet (UV) disinfection emerges as an interesting alternative to be applied in these communities. Capable of inactivating bacteria, viruses, and protozoa, the UV treatment offers the advantage of not altering the taste and odor of drinking water, unlike chlorine. Nonetheless, the possibility of pathogen regeneration through photoreactivation and dark repair after UV disinfection poses risks during the storage and consumption stages over time (Wang, 2021).

Thus, to ensure safely treated water by UV-based systems without combined chemical products, it is crucial to understand the mechanism of bacterial regrowth in the post-UV disinfection phase, as well as the possibility of controlling regrowth through induced cellular damage by radiation dosage (Wang, 2021; Zhang et al., 2023). Hence, this study aimed to verify, on bench scale, the possibility of minimizing bacterial regrowth by increasing the UV radiation dose applied to the disinfection of groundwater.

METHODOLOGY

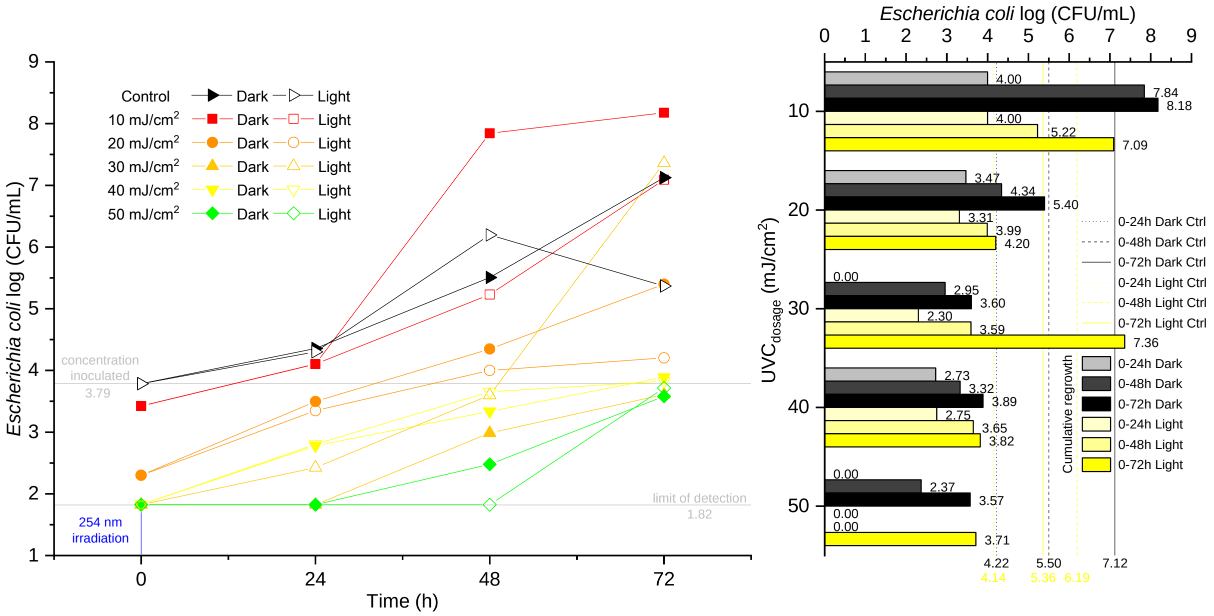
The influent water used in this study (i.e., general test water or GTW by WHO, 2018) simulated a groundwater source (pH of 7.0 ± 0.5, TOC of 1.05 ± 0.95 mg/L, turbidity ≤ 1 NTU) contaminated with 6×103 CFU/mL of *Escherichia coli*.

20-mL samples were exposed to radiation in an UV-collimated beam device (λ = 254 nm with 0.209 mW/cm²) under doses of 10, 20, 30, 40, and 50 mJ/cm². After each UV exposure dose, 10 mL aliquots were collected and transferred to two previously sterilized Falcon tubes. One remained exposed to indirect sunlight and the other remained in the dark. In both cases, aliquots were taken at 0 hours and after 24, 48, and 72 hours and microdiluted in phosphate-buffered saline for microbiological analysis. *E. coli* colonies were counted using the Chromocult Coliform Agar® at 37°C for 21 ± 3h. The detection limit (DL) was 67 UFC/mL (1.82 in Log_{10}).

RESULTS AND CONCLUSIONS

The UV radiation decreased the *E. coli* concentration from 6×103 CFU/mL to below the detection limit (DL) of the quantification method in doses higher than 30 mJ/cm^2 (i.e. Figure 1a, at 0 h in blue). In UV exposures of 10 mJ/cm^2 and 20 mJ/cm^2, the remaining *E. coli* values were 3×10^3 CFU/mL and 2×10^2 CFU/mL, respectively. Hatano et al. (2023), who observed a logarithmic decline in disinfection efficacy, particularly for doses exceeding 36 mJ/cm^2, supported these findings.

Although UV doses of 30, 40 and 50 mJ/cm^2 achieved *E. coli* below the DL, the increase up to 50 mJ/cm^2 demonstrated an effect on minimizing the regrowth over time. Moreover, the 50 mJ/cm^2 dose, where the sample was indirectly exposed to sunlight (Figure 1a, in green), was the only one that showed no regrowth within 48 hours, but a peak of 3.71 log at the end of 72 hours. The same phenomenon was observed by Nyangaresi et al. (2019) and Hatano et al. (2023), suggesting that *E. coli* remains in a latency period due to a residual effect of UV radiation on bacterial DNA and subsequently resumes significant growth. Another notable observation was the dark repair phenomenon in *E. coli* after UV irradiation, suggesting that the latency period may also be prolonged in experiments with indirect sunlight exposures. For example, while the 50 mJ/cm^2 sample kept in the dark exhibited a regrowth of 2.37 log within 48 hours, the sample exposed to sunlight remained below the DL (see Figure 1b, 0-48 h Dark and Light).



(a) (b)

Figure 1 – Regrowth of *E. coli* from 0 to 72 hours (a) and cumulative regrowth at 24, 48 e 72 hours (b).

An unexpected result was observed in samples exposed to 10 mJ/cm², in which there was *E. coli* regrowth at 72 h higher than the concentration inoculated in the study water and the control assay (Figure 1a, b). A possible explanation is that dissolved organic matter, such as residual concentrations of tryptone, sodium pyruvate, and fulvic acid, along with TOC, have an influence on the regrowth of *E. coli* after UV exposure (Hatano et al., 2023).

In conclusion, the observations suggest that increasing the radiation dose, whether maintained at a higher or lower intensity, such as artificial radiation (i.e., UV lamps and LED-UV diodes) and natural radiation (i.e., direct and indirect sunlight), may act as maintenance of the latency state of *E. coli* after primary irradiation. Additionally, the presence of organic substances poses risks to water storage post-treatment. Therefore, the UV radiation dose and/or maintenance dose (radiation in the reservoir), coupled with the control of organic substances in groundwater sources, may be key factors in managing the risk of bacterial regrowth in reservoirs of water disinfected solely with UV.

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