Effect of Radiation Intensity and Storage Conditions on Bacterial Regrowth Patterns

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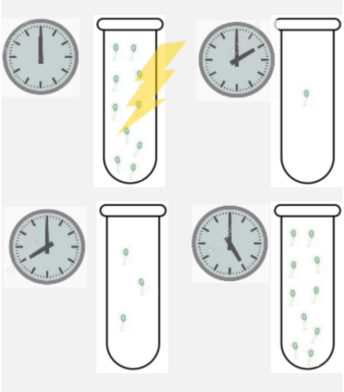

The use of UV radiation has proven to be a highly effective method for disinfecting a multitude of microorganisms. However, the lack of residual effect of this type of treatment and the impossibility of ensuring total disinfection opens the door for bacterial regrowth. In this work, the bacterial regrowth of the *E. coli* bacteria was studied depending on different parameters, such as the radiation treatment dose or the subsequent storage conditions. The results showed that high doses of UVC radiation inhibit subsequent regrowth. Furthermore, it was possible to observe how the storage of the treated water at temperatures close to the optimal for bacterial growth favored the darkness regrowth phenomena.

Introduction

Sustainable Development Goal 6 (SDG 6) centers on securing water and sanitation availability, managing them sustainably for global populations. This goal addresses challenges arising from factors like population growth, rapid urbanization, economic development, and changing consumption patterns. Additionally, the impact of climate change and extreme weather events, such as droughts and floods, further complicates meeting water demands. The United Nations General Assembly acknowledged the human right to water and sanitation in 2010, underscoring its importance for overall human rights fulfillment. This right asserts that everyone should have continuous access to sufficient, healthy, physically reachable, affordable water of acceptable quality.

Waterborne microorganisms, including viruses, bacteria, and protozoa, pose severe health risks, contributing to the spread of diarrheal diseases. According to the World Health Organization, water-related diseases like diarrhoea, cholera, dysentery, typhoid, and polio lead to over 3.4 million deaths annually.

A successful water disinfection method involves utilizing UVC radiation. This technique irradiates water with UVC light, damaging the genetic material of bacteria and viruses and disrupting their reproduction systems [1]. However, a notable drawback compared to other methods like chlorination is that UVC irradiation doesn't leave a residual disinfectant in the water, leaving it vulnerable to subsequent contamination. The phenomenon of increased bacterial population after disinfection has been extensively documented [2].

UV disinfection works by directly damaging bacterial DNA or inducing internal oxidative stress. Shortwave UV irradiation causes DNA lesions, disrupting replication and transcription. Microorganisms can repair this damage through photoreactivation and dark repair mechanisms. Photoreactivation involves exposure to specific light wavelengths, enabling DNA photolyases to break down dimers formed under UV exposure. Dark repair mechanisms use enzymes to break DNA cross-links without requiring light energy. If complete inactivation isn't achieved, bacterial populations may increase in water with sufficient nutrients due to the surviving bacteria's multiplication capacity. This differs from bacterial reactivation from the non-culturable viable state, as this fraction remains viable and culturable throughout the process. Hence, the presence of viable non-culturable cells, DNA-damaged cells, and intact cells in disinfected water always indicates the possibility of observing new growth after disinfection [3].

Understanding the treatment's effectiveness and regrowth mechanisms after disinfection is crucial for determining the optimal storage conditions and reliable duration for treated water. This study specifically explores the regrowth of *E. coli* bacteria after treating contaminated water with UVC LED, examining the influence of UVC dose, initial concentration, and storage conditions.

Material and Methods

For the inactivation experiments, a quartz tube with an outer diameter of 23 mm and an inner diameter of 20 mm served as the experimental reactor. The circulation of water from a buffer tank, configured in a single-pass manner, occurred through the quartz tube. A centrifugal pump with a flow rate of 2.85 L/min facilitated the water flow. The irradiation source utilized was LED with a wavelength of 265 nm (KL265-50U-SM-WD, Klaran), integrated into a

COBRA Clean FX-1 system from ProPhotonix IRL. Four similar lighting systems, each incorporating 16 UVC LED, were employed. The active irradiated volume within this setup measured 28.27 cm^3. Irradiance in water was measured using ferrioxalate actinometry, following the procedure outlined by [4], in recirculation mode, as the technique required extended exposure times for incident irradiation calculations.

Escherichia coli K12 strains, suspended in distilled water with 0.9% NaCl, were used for disinfection tests. Fresh liquid cultures were prepared through inoculation into Luria-Bertani (LB) nutrient medium (Miller's LB Broth, Scharlab) and incubated at 37°C for 24 h with constant shaking on a rotating shaker. Sample analysis followed a standard serial dilution procedure, with counting of viable bacterial colonies performed after 24 h of incubation at 37 °C. Additionally, The study of total aerobic bacterial regrowth in an effluent from the secondary treatment of a WWTP after disinfection was also studied.

The water samples post-disinfection were preserved under various conditions to investigate bacterial regrowth, examining the impact of temperature and the presence or absence of light. The quantification of viable bacterial colonies was revisited after 1, 2, and 7 days from the disinfection process.

Results and Discussion

Disinfection experiments for two different radiation intensities: $3.21\cdot10^{-6}$ and $2.41\cdot10^{-6}$ E/s, equivalent to 100% and 75% of the maximum light intensity of the system, respectively, are shown in the figure. It is noteworthy that immediately following irradiation in both cases, no bacterial colonies were observed. However, it is essential to emphasize that the absence of viable bacteria does not indicate complete disinfection; rather, it signifies the non-observation of bacteria using the employed analysis method.

When examining bacterial resurgence in the presence of light, it could see that bacterial concentrations remained negligible in both instances during the initial two days. However, a remarkable rebound to nearly 40% of the initial concentration occurred after 7 days in the experiment with the lowest radiation intensity. Conversely, the highest radiation intensity showed no signs of regrowth under illuminated conditions over the 7-day period. The collected data highlight the substantial impact of radiation dosage on subsequent bacterial regrowth, indicating that higher doses may contribute to a diminished regrowth rate, potentially due to more extensive damage inflicted.

When storage was conducted in dark conditions, it was observed that for both doses, bacterial regrowth of approximately 20% occurred from the first day post-treatment, eventually reaching bacterial concentrations comparable to the initial levels after 7 days. These results indicate that photoreactivation played a less significant role compared to repair processes in the absence of light. This finding could be attributed to the use of a storage temperature close to the optimal range for bacterial growth [4].

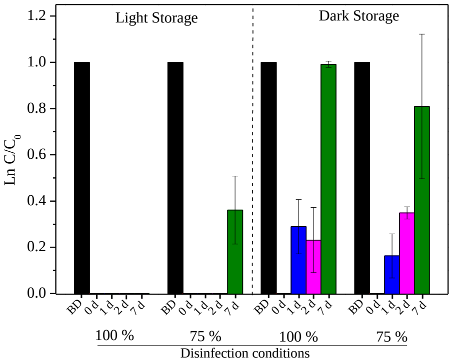

Figure 1. Bacterial regrowth after disinfection with doses of 75 and 100% ($2.41\cdot10^{-6}$ and $3.21\cdot10^{-6}$ E/s, respectively) of the maximum over 7 days of storage at 37ºC under light and dark conditions.

Conclusions

Disinfection methods based on UV radiation are effective for removing pathogens from water, however, the lack of residual effect causes its regrowth, making it impossible to store treated water for prolonged periods of time. The regrowth rate is influenced by the radiation dose received as well as the storage conditions so its study is crucial to determine safe period of water consumption.

Acknowledgments

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