

Evaluation of Photoreactivation and Dark Repair of Total and Fecal Coliforms and Enterococci in Wastewater Treated with Ultraviolet Light

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Abstract Wastewater treatment plants are essential in reducing the microbial load of water discharged into the ecosystems by UV light. However, it has been found that some pathogenic bacteria have developed mechanisms to reverse the damage caused to their DNA by UV light, with possible adverse effects on the environment and human health. Therefore, this research evaluated if fecal indicator bacteria that use photoreactivation or dark repair are present in the wastewater from a treatment plant in Puerto Rico. Samples of wastewater treated with UV light were collected and exposed to two treatments: fluorescent light (photoreactivation) and darkness (dark repair). The number of colonies of total coliforms, fecal coliforms, and enterococci was determined every hour of exposure. Results show that after exposure to fluorescent light, the number of colonies of total and fecal coliforms increased, being able to repair and reverse the damage caused to their DNA when exposed to visible light but not in darkness, possibly through the mechanism of photoreactivation. However, enterococci showed no increase in colonies when exposed to fluorescent light and kept in darkness. These results suggest reviewing the disinfection process considering photoreactivation and dark repair mechanisms. The new considerations can reduce pollution of watersheds when large amounts of treated wastewater are released into the environment.

Keywords: bacteria, sewage, disinfection, water, enterobacteria

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1. Introduction

One of the current environmental problems is the poor control to prevent water bodies from becoming contaminated by several sources (e.g., wastewater). In Puerto Rico, wastewater must comply with effluent guidelines and quality levels established by the Environmental Protection Agency (EPA) [1]. The main objective is to ensure that human and industrial effluents are discharged safely into water bodies and do not represent a risk to human health or unacceptable environmental damage [2].

Treatment plants use conventional ultraviolet light (UV) to disinfect the effluents before discharge into the receiving waters [3]. UV disinfection works by damaging the microorganism's nucleic acid, preventing its replication [4]. This method does not leave residuals in the water after treatment, which is an advantage over chemical disinfection [5].

Nonetheless, bacteria can repair DNA damage caused by UV light, possibly reactivating their activity after the water leaves the treatment plant [3,6]. It has been reported that microorganisms can recover the biological damage caused by UV radiation by using photoreactivation [5]. Photoreactivation has been reported in microorganisms from wastewater treated with UV light and after exposure to sunlight when discharged to the basins [7]. This light-dependent DNA repair mechanism happens when the microorganism produces the photolyase enzyme. This enzyme is responsible for repairing DNA damage when the bacteria are exposed to light, as it effectively reverses the harmful effects in the genome caused by UV radiation [8,9]. As a result, this could reduce the effectiveness of the disinfection process, which is a risk to public health and the water body's health.

Bacteria may also use a dark repair mechanism to repair the damage induced to their DNA by the action of several enzymes without the need for specific light [10]. This mechanism uses various DNA repair processes that do not depend on the light; they require that the *uvrA*, *uvrB*, and

uvrC genes initiate the repair of pyrimidine base dimers (cyclobutane pyrimidine dimers) and photoproducts of 6-4, as well as other voluminous lesions [11]. Nucleotide excision repair (NER) has also been proposed as the pathway to repair UV-damaged DNA without exposure to radiation [12]. However, some research concludes that dark repair occurs less than photoreactivation [13].

Besides being necessary, wastewater technologies and processes must be adequate to protect aquatic ecosystems [14]. This work aimed to evaluate if photoreactivation and dark repair occur in total coliforms, fecal coliforms, and enterococci in wastewater treated with ultraviolet light in Puerto Rico. Therefore, UV-treated wastewater samples were exposed to fluorescent light or kept in darkness, and the growth of those organisms was monitored.

2. Methodology

2.1. Water Collection

Wastewater samples were collected in 500 mL sterile plastic bottles, on three different occasions, from a treatment plant on the west side of Puerto Rico. This secondary wastewater plant removes biodegradable organic matter and utilizes UV light as a disinfection method. After the disinfection, the samples were taken at the final stage before the effluent reached a municipal ravine that joins the Güanajibo River in Puerto Rico. All the samples were transported under refrigeration conditions to the laboratory.

2.2. Evaluation of Repair Mechanisms by Ultraviolet Radiation



Figure 1. Erlenmeyer flasks exposed to fluorescent light and darkness in a controlled environment

The water samples were divided and transferred to seven 1,000 mL Erlenmeyer flasks (with 95% transparency). Three flasks with 1,000 mL of water sample were designated to evaluate the light exposure, and the other three flasks were covered with aluminum foil to simulate darkness conditions. The seventh Erlenmeyer flask was used as a control for the experiment. All the Erlenmeyer flasks were placed in a controlled environment incubator equipped with a fluorescent bulb (Ecosmart 19 watts) at a 4 cm distance from the flasks and

a temperature of 26.5°C (Figure 1). The irradiation periods with fluorescent light were up to 300 minutes in total coliforms, and for fecal coliforms and enterococci were up to 240 minutes. An Erlenmeyer flask with 1,000 mL of sterilized sample was used for negative control.

The concentration of total coliforms, fecal coliforms, and enterococci was determined for microbiological contamination. The EPA standard method 1603 for examining enterobacteria in water using the membrane filtration technique (0.45µm) was used [15]. 1 mL was filtered for total coliforms; 50 mL for fecal coliforms; and 100 mL for enterococci; every 60 minutes (starting at time 0) until reaching 300 minutes for total coliforms and 240 minutes for fecal coliforms and enterococci to evaluate repair mechanisms.



Figure 2. Total coliforms colonies in m-Endo agar after exposure to light (A) and kept in darkness (B)

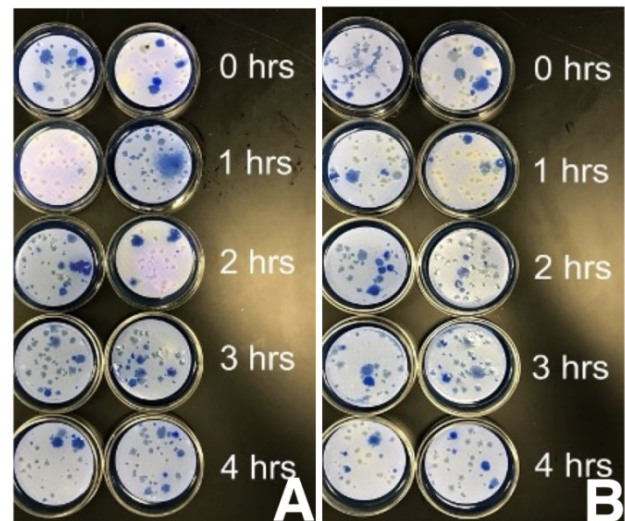


Figure 3. Fecal coliforms colonies in m-FC agar after exposure to light (A) and kept in darkness (B)

Total coliforms were cultured in m-Endo agar and incubated at 35°C for 24 hours (Figure 2). Next, fecal coliforms were cultured in m-FC agar for 24h of incubation at 44.5°C (Figure 3). Finally, enterococci were cultured in m-Enterococcus agar at 35°C for 48 h

(Figure 4). After the incubation period, the number of bacterial colonies was determined, and the colony-forming units (CFU) per 100 ml sample were calculated. This experiment was carried out in triplicate with duplicates, and average values of CFU were obtained.

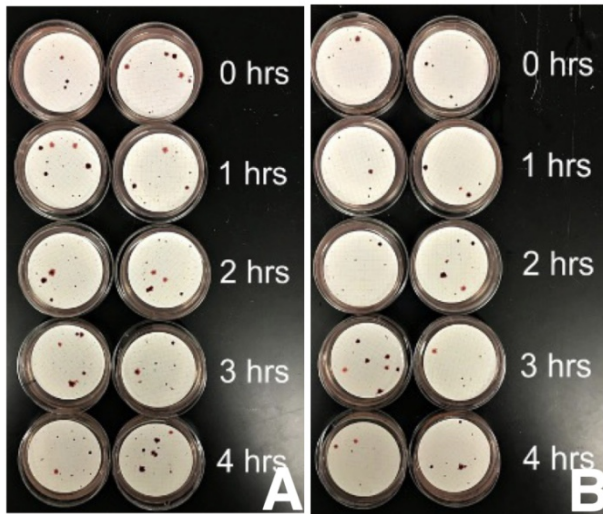


Figure 4. Enterococci colonies in m-Enterococcus agar after exposure to light (A) and kept in darkness (B)

The relation N/N_0 [10] was used to determine the relationship between the concentration of colonies of total coliforms, fecal coliforms, and enterococci present after being treated with ultraviolet light. Where N represents the number of colonies present after exposure to visible light or darkness, and N_0 represents the number of colonies before exposure to light or darkness. Also, colonies' concentration of each group of bacteria as a function of time under visible light and darkness conditions were determined.

Traditional aseptic transfer techniques and differential staining methods were used to determine the microscopic characteristics of the microorganisms evaluated. The colonies observed in the selective-differential culture media used for this analysis were transferred to inclined agar tubes to determine by staining the microscopic characteristics of the microorganisms. For this, a Gram stain was used, differentiating between two large groups

of bacteria (Gram-positive and Gram-negative) based on their cell wall composition.

3. Results and Discussions

After UV treatment in the wastewater plant, the growth of some bacteria is not expected because of the DNA damage occasioned by UV light exposure. However, under the conditions of this experiment, total coliforms exposed to visible light grew despite being previously exposed to UV light. Furthermore, our results showed an increase in N/N_0 when exposed to visible light for up to 5 hours (Figure 5). This increase reflects that there were colonies capable of repairing and reversing the damage caused to their DNA by the UV treatment when later exposed to visible light, demonstrating a possible mechanism of photoreactivation. Previous works have also reported the capability of this group of bacteria to resist and repair the harmfulness caused by UV rays [13,16,17,18,19]. However, none have been reported in Puerto Rico.

On the other hand, total coliforms did not grow in darkness, so dark repair was not evident. In investigations where this mechanism has been studied in total coliforms, it has been found that dark repair occurs considerably lower than in photoreactivation [13]. However, over time a reduction in the number of colonies occurs, and when the final count is compared with the initial count, it is concluded that this mechanism is irrelevant. The results of this study are like other investigations, in which no significant differences are found in the growth of TC in darkness [6].

Fecal coliforms also grew by exposing them to visible light after being treated with UV light treatment (Figure 6), reversing the damage caused to their DNA, possibly by a photoreactivation mechanism. The results obtained can be compared with other investigations [10,13,20,21] that reported that fecal coliforms and *Escherichia coli* (fecal coliform) demonstrated the ability to carry out photoreactivation by exposure to visible light after being exposed to UV light. In addition, the DNA repair under light exposure has been explained by the presence of the enzyme photolyase in the bacterium *E. coli* [16].

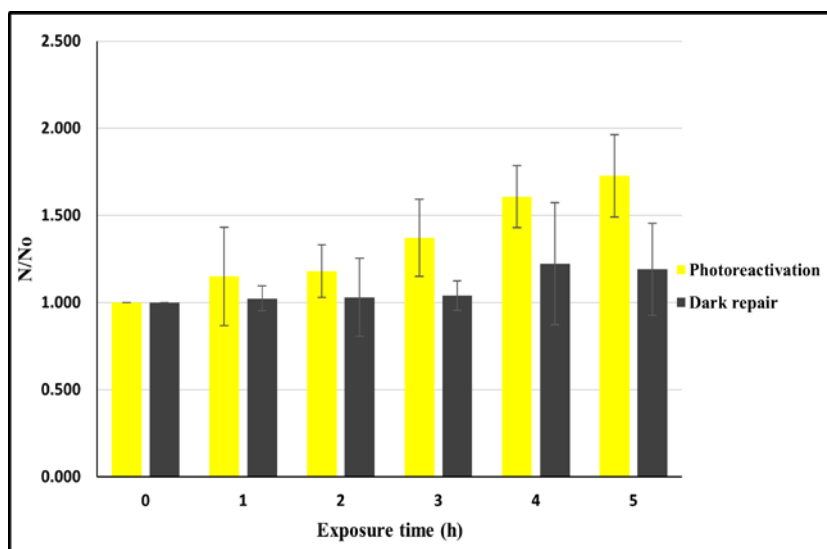


Figure 5. Comparison of N/N_0 of TC exposed to visible light (photoreactivation) and darkness (dark repair)

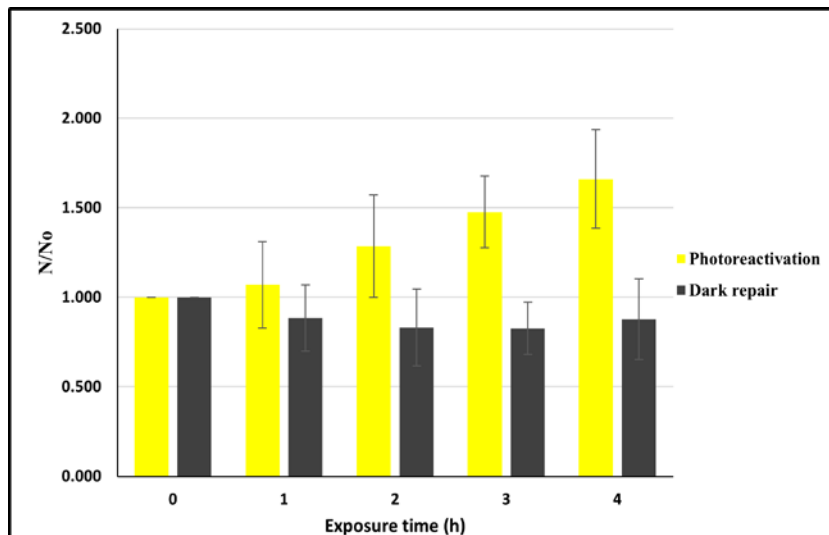


Figure 6. Comparison of N/No of FC exposed to visible light (photoreactivation) and darkness (dark repair)

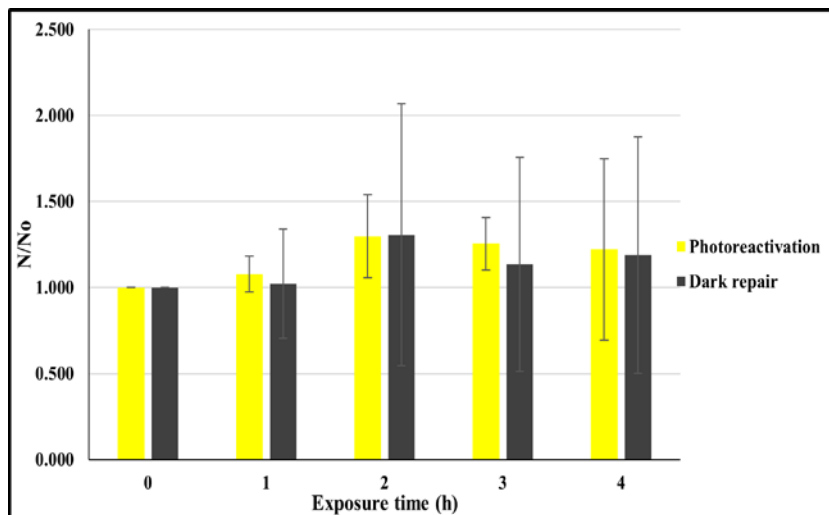


Figure 7. Comparison of N/No of EC exposed to visible light (photoreactivation) and darkness (dark repair)

Like total coliforms, fecal coliforms did not make the mechanism of dark repair evident. On exposure to darkness, the proportion of the number of colonies was statistically the same at all times evaluated. The results obtained in dark repair can be compared with other investigations [10,13,20], showing limited or no repair in this group of bacteria when kept in darkness.

When evaluating the effect of darkness on the growth of enterococci, some studies reported that they are capable of photoreactivation [13,22], but this was not the case in our investigation. The N/No ratio was similar from the beginning to the end of the exposure period. Our results for enterococci showed that they could not repair themselves in treatment with visible light or darkness (Figure 7). Nonetheless, this is consistent with other studies that report that enterococci do not carry out any repair mechanism [10,23].

4. Conclusions

Our research proved that the total and fecal coliforms in Puerto Rico wastewaters have photoreactivation mechanisms in light. On the contrary, the enterococci group did not

show growth related to photoreactivation processes. Furthermore, when kept in darkness, none of the three groups of bacteria show growth, which could indicate the dark repair of damaged DNA. These results suggest reviewing UV disinfection processes in wastewater treatment plants to determine if the pathogenic microorganism is reactivating after water is released into the environment. This review is essential for Puerto Rico, where light and water temperatures may be suitable for those species' growth, reducing and avoiding pollution of watersheds when large amounts of treated wastewater are released into the environment.

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