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Performance Parameters: Demobilization Antibiotic Resistant Bacteria (ARB) and Carrying Genes (ARG) in Wastewater Disinfection

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Abstract: The UV irradiation is used for removing Antibiotic Resistant Bacteria (ARB) and Antibiotic Resistance Genes (ARG) from wastewater treatment. Bacteriophages are viruses that infect within bacteria, are recognized for bacterial control. The influence of some parameters in quantification and performance influencing of pathogen demobilization could be considered in disinfection of wastewater. The comparison of Polyvalent phage (NE1) versus Coliphage (NE4) in suppressing a bacterium *Escherichia coli* (NDM-1: *β-lactam-resistant)* with UV irradiation was observed the efficacy in reduction of cells in the disinfection and parameter process. The results with the effect of UV-C irradiation on NDM-1 infected with 1 % of NE4 showed a decrease of cells from 8 x 10^6 to 2 x 10^5 in 60 min with UV-C dose. The NDM1 (*E. coli*) was infected with 1 % of NE4 (Polyvalent Phage) under magnetic stirring for 1 h, the cells count was 8 x 10^6. After 1 h in UV-C exposure, the cells number reached 3 x 10^5. The NDM1 that was exposed in 1 h of UV-C irradiation and then was infected with 1 % of NE4. Cells counting were done 24 h after this procedure. These cells were exposed in UV-C and showed a reduction in the number of cells from 1 x 10^8 to 4 x 10^5 after 60 min. The results indicate that bacteriophages can mitigate bacteria species, and combined the conventional water disinfection technologies that can support the microbial safety control strategies.

Key words: Antibiotic Resistant Bacteria (ARB), Antibiotic Resistance Genes (ARG), wastewater treatment, disinfection, *Escherichia coli (E. coli)*.

1. Introduction

Antibiotics have been used for the prevention and treatment of infectious diseases [1]. They have saved people's life, but the abuse of antibiotics and the discharged into the environment have emerged of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) [2]. The propagation of infection by superbugs has potential risk to human health and ecological environmental [3].

Wastewater treatment provides suitable conditions for ARB reproduction and ARG horizontal gene transfer due to the plenty nutrients, particular pressures influenced by pollutant compounds such as toxic metals, biocides and antibiotics [4]. The environmentally relevant microbiota is run into wastewater treatment plants (WWTP), assisting the partition of ARG among them, and introducing resistance mechanisms to ARB [5]. The treatment plants are planned to remove pathogenic microorganisms nor even to eliminate ARG, and diverse ARB are present in treated wastewater [6].

Disinfection with ultraviolet irradiation (UV) is commonly used in wastewater treatment for eliminating and controlling the spread of ARB and ARG present in

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effluents [7]. Different from chlorination, which is also another commonly treatment approach for controlling distribution of bacteria, UV irradiation treatment inactivate bacteria within a short time and without the production of undesirable toxic by-products [8].

Bacteriophage in microbial control studies could assist to attenuate the proliferation of antibiotic-resistant bacteria ARB in wastewater treatment. Horizontal gene transfer is an important step for competitive bacterial survive in wastewater and is a driver for ARG transfer. Plasmid based conjugative transfer has been studied under laboratory conditions. ARG enhance the spread of non-resistant bacterial groups by horizontal gene transfer and plasmid conjugative processes. Studies have investigated the influence of disinfection on ARB and ARG by UV disinfection procedure that may be possible to remove ARB and ARG [9, 10].

In this study, a novel approach to mitigate ARB proliferation in wastewater based on the use of UV in combination with bacteriophages treatment was applied. For it, a nonpathogenic host, *Escherichia coli* NDM-1 (*β-lactam-resistant)* was used to grow the coliphage NE4 and the polyvalent phage NE1 was investigated. Polyvalent Phage NE1 was isolated from activated sludge versus coliphage NE4 (Carolina Biological 12-4330) in suppressing a bacterium NDM-1 (β-lactam-resistant *E. coli*) [11]. The combination of a bacteriophage and UV irradiation presented interesting results to improve the wastewater treatment offering new perspectives for more tests and use.

2. Methods and Data

2.1 Bacteria, Bacteriophage, and Culture Conditions

The bacterial strain used in this study included *E. coli* NDM-1 carrying the plasmid-encoded $bla_{\text{NDM-1}}$ gene is resistant to β-lactam antibiotics. Culturing was conducted in LB broth at 37 ℃ (10 g of Tryptone, 5 g of Yeast Extract, per liter water supplemented with 10 g of NaCl, and adjusted pH 7 with solution of NaOH, 1 N. Coliphage NE4 (Carolina Biological 12-4330) and the polyvalent Phage NE1 was previously isolated by a sequential multi host approach. Bacteria strain in exponential phase were used for phage treatment. Double-layer plaque assays used Soft Agar solution (4 g of Bacto Tryptone, 3.6 g of Agar Powder, 2 g of KCl, per 400 mL water supplemented). Tryptone agar plates containing 0.7 % agar for the soft agar and 1.1 % for the base layer. Phage was performed using NDM-1 as the host and expressed as plaque forming units (PFU) per milliliter. The initial concentration of bacteria host NDM-1, coliphage NE4 and polyvalent NE1 were 7×10^7, 1.8×10^{10} and 3×10^9, respectively.

2.2 UV Disinfection Experiment

A laboratory apparatus composed by a lamp of low-pressure mercury emitting UV light at 245 nm (120 W, Sankyo Denki Ltd. Tokyo, Japan) was used which has previously demonstrated to effectively damage cell membrane [12]. The organism samples, well mixed with a magnetic stirrer in petri dishes and maintained at room temperature (25 ± 5 ℃). The experiments were performed using a selected UV-A and UV-C doses controlling the exposure time. After the disinfection period, a duplicate of 1 mL of the UV exposed sample was removed from the petri dish, and disposed on a double-layer plaque on a solution composed by PBS (phosphate saline buffer) and SMB (Basic salts) medium (5.8 g of NaCl, 1.2 g of MgSO4, 50 mL of Tris HCl pH 7.5 (1M)), and per liter water supplemented with 1 g of gelatin). The plaques were incubated at 35 ℃ for 48 h. Enumeration of waterborne organisms has been carried out using plate count methods.

3. Results

3.1 Effect of UV-A Irradiation Exposure Time on NDM-1, Coliphage NE4 and Polyvalent Phage NE1

Samples containing NDM-1, Coliphage NE4 and Polyvalent Phage NE1 was irradiated with UV-A in 30 and 60 minutes decreased as shown in Fig. 1.

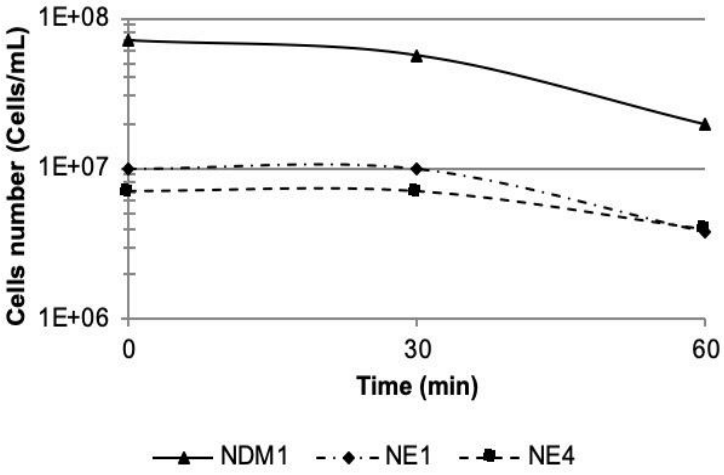

Fig. 1 Temporal variation of total bacteria (cells count) exposed to UV-A dose in NDM-1 (E. coli), NE4 (Coliphage) and NE1 (Polyvalent Phage).

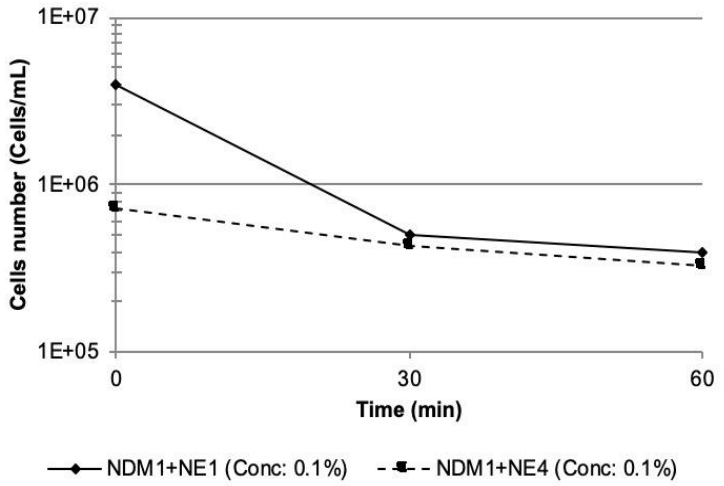

Fig. 2 The cells number of bacteria exposed to UV-A in NDM-1 (*E. coli*) infected with 0.1 % of NE4 (Coliphage) and NE1 (Polyvalent Phage).

The effect of phages in 0.1 % concentration on UV-A disinfection efficiency in NDM-1 as shown in Fig. 2. This experiment was used NDM-1 infected with NE1 in concentration of 0.1 % and NDM-1 infected with NE4 in the same concentration (0.1 %). Fig. 2 shows the effect on cells of NDM-1 infected with NE1 and NE4 irradiated by UV-A dose during a maximum of 1 hour.

Fig. 3 shows the cells count of NDM-1 with 1 % of NE1 and NE4 in exposure of UV-A light for a total of 1 h.

Fig. 4 shows experiments with NDM-1 infected with 10 % of NE1 and NE4 in UV-A exposure for 1 h.

3.2 Effect of UV-C Irradiation on NDM-1, Coliphage NE4 and Polyvalent Phage NE1

Fig. 5 shows the effect on cells count with UV-C exposure on NDM-1 (*E. coli)*, NE4 (Coliphage) and NE1 (Polyvalent Phage).

The result showed the effect of UV-C dose on NDM-1 infected with 1 % of NE1 and NE4. Fig. 5 and 6 show the cells count of NDM-1 exposed in UV-C light for 1 h with 1 % of concentration of NE4 and NE1.

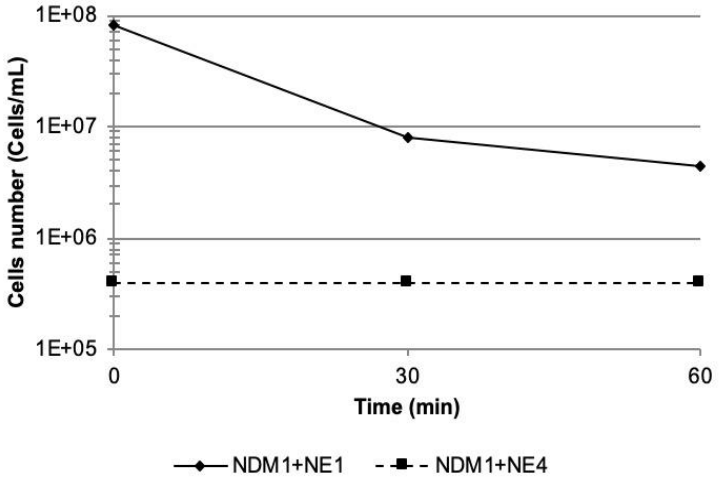

Fig. 3 Temporal variation of cells number exposed to UV-A in NDM-1 (*E. coli*) infected with 1 % of NE1 (Polyvalent Phage).

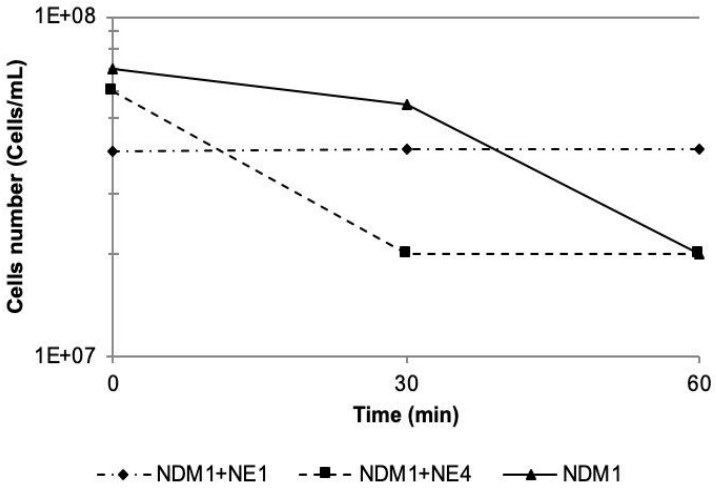

Fig. 4 The cells count exposed to UV-A in NDM-1 (*E. coli*) infected with 10 % of NE4 (Coliphage) and NE1 (Polyvalent Phage).

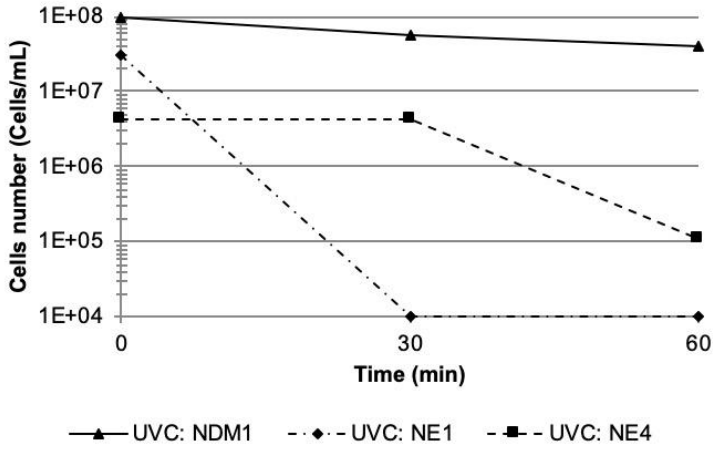

Fig. 5 Temporal variation of total bacteria (cells count) exposed to UV-C in NDM-1 (*E. coli*), NE4 (Coliphage) and NE1 (Polyvalent Phage).

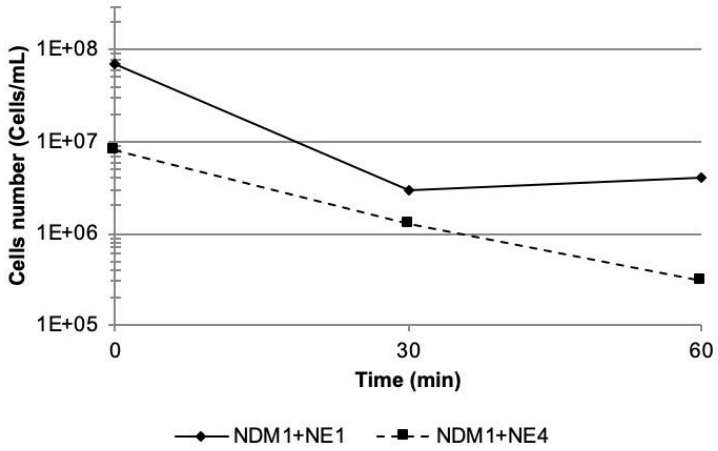

Fig. 6 The variation of total bacteria (cells count) exposed to UV-C in NDM-1 (*E. coli*) infected with 1 % of NE1 (Polyvalent Phage).

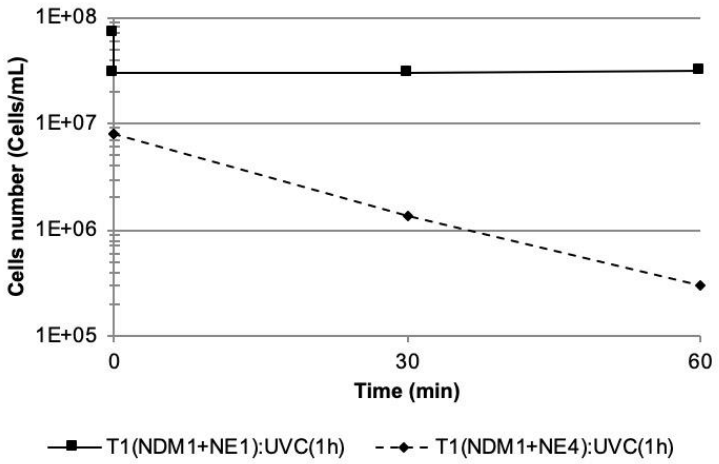

Fig. 7 Test 1 (T1): Temporal variation of total bacteria (cells count) exposed to UV-C in NDM-1 (*E. coli*) infected with 1 % of Coliphage under 1 h of agitation.

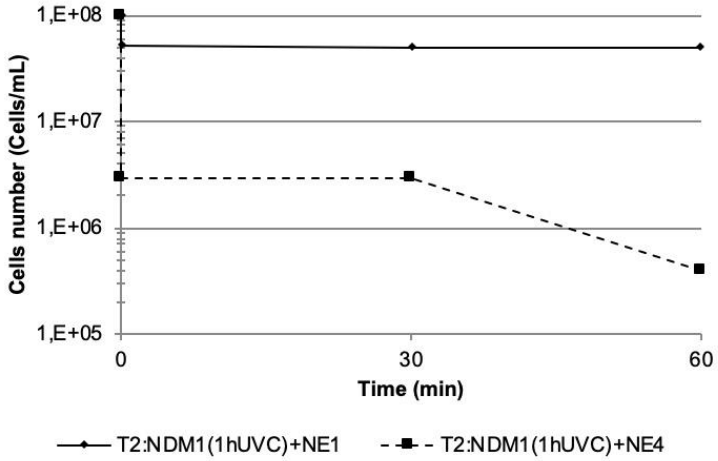

Fig. 8 Results of the Test 2 (T2): The NDM-1 (*E. coli*) exposed in UV-C dose for 1 h. After this procedure, NDM-1 was infected with 1 % of Coliphage.

3.3 UV-C Disinfection Performance: Agitation Parameter

Fig. 7 shows the cells count of the Coliphage at a concentration of 1 % hosted in NDM-1 under magnetic stirring for 1 h and then the cells were exposed in UV-C light for 1 h (Test 1-T1).

Fig. 8 shows that the UV-C exposure of NDM-1 for 1 h and after this the experiment was infected with 1 % of Coliphage (Test 2-T2).

4. Discussion

4.1 Effect of UV-A Irradiation Exposure Time

Fig. 1 showed the effect of UV-A light on host NDM-1 infected with Coliphage NE4 and Polyvalent Phage NE1. In 30 minutes of exposure to UV-A negligible reductions in bacteria and phages concentrations were observed. Increasing exposure to 60 min. led to a measurable reduction of NDM-1 from 7 to 2 x 10^7 CFU/ mL, NE1 from 1 to 0.5 x 10^7, and NE4 from 0.8 to 0.5 x 10^7.

The experiment in Fig. 2 shows the cells count of NDM-1 infected with NE1 at 0.1 % of concentration in first time with 4 x 10^6. The cells number decline significantly after 30 min. of UV exposition (5 x 10^5 cells) and then showed a plateau in 60 min. of UV dose (4 x 10^5 cells). The cells of NDM-1 infected with 0.1 % of NE4 showed a decrease of cells from 7 x 10^5 to 4 x 10^5 cells with 30 min. of UV-A dose and decrease to 3 x 10^5 cells with 60 min of UV-A light exposure.

Fig. 3 shows a decrease in the cells with 1 % of NE1 from 8 x 10^7 to 8 x 10^6 with 30 minutes in UV-A exposure and then reaching 4 x 10^6 in 1 h of UV-A exposure. The cells of NDM-1 with 1 % of NE4 were rest constant in 4 x 10^5 cells. In this experiment the UV-A exposure presented no effect in cells number NDM-1 with 1 % of NE4.

Fig. 4 shows that the cells of NDM-1 with 10 % of NE1 rest constant in 4 x 10^7 with 30 min. and 1 h of UV-A dose. The UV-A exposure presented no effect in this experiment. The cells of NDM-1 with 10 % of NE4 started with 6 x 10^7 and after 30 min. in UV-A irradiation decreased to 2 x 10^7 cells and rest constant with 1 h in UV-A light exposure.

4.2 Effect of UV-C Irradiation

Fig. 5 shows that NDM1 declined in the number of cells in 30 min. of UV-C dose, and then, there was a significantly decrease from 1 x 10^8 to 5.8 x 10^7. In the final of irradiation by UV-C exposition (60 min.), the cells number declined to 4 x 10^7. It was evidenced that bacteria number was affected by UV-C irradiation.

The inactivation rate increased with increasing UV-C dose. Coliphage NE4 shows a plateau in the number of cells in the first time of 30 min UV-C dose (4 x 10^6). After that the number of cells decreased to 1 x 10^5 in 60 min UV-C dose. Polyvalent Phage NE1 shows a decrease in the number of cells in the first time of 30 min in UV-C dose from 3 x 10^7 to 1 x 10^5. After that the number of cells kept constant to 1 x 10^5 with 60 min of UV-C dose.

Fig. 6 shows that cells of NDM1 infected with 1 % of NE1 declined in the number of cells in 30 min. of UV-C dose, and then there was a significant decrease from 7 x 10^7 to 4 x 10^6 cells number. In the final of irradiation by UV-C exposure (60 min.), the number of cells kept constant to 4 x 10^6. The inactivation rate increased with long time of UV-C exposition. NDM-1 with 1 % of Coliphage NE4 shows a decrease in the number of cells in 30 min. UV-C exposition varying from 8 x 10^6 to 1 x 10^6. After that the number of cells decreased to 2 x 10^5 in 60 min.

4.3 UV-C Disinfection Performance

Demeersseman et al., 2023 inform an overview of performance-influencing parameter in UV-C systems. The authors showed some parameters such as the wavelength, dose, humidity and temperature [13]. In this experiment (Fig. 7) showed the results under magnetic stirring in the process. The NDM-1 (*E. coli*) was infected with 1 % of NE1 (Polyvalent Phage) under magnetic stirring for 1 h, the cells count was 7 x 10^7 and

decreased to 3 x 10^7 and rest constant after 1 h of UV-C exposure.

Fig. 7 shows that during the infection of 1 % of NE4 (Coliphage) in NDM-1 (*E. coli*), the number of cells remained constant at 8 x 10^6 and then, in 30 min. of UV-C exposure, it showed a decrease of the number of cells in 1.3 x 10^6. After 1 h in UV-C exposure, the cells number reached 3 x 10^5.

Fig. 8 shows UV-C exposure of NDM-1 for 1 h and after this it was infected with 1 % of NE1. Cells counting were done 24 h after this procedure. The procedure showed a reduction in the number of cells from 1 x 10^8 to 5 x 10^7 and remained constant during the entire processes (1 h in UV-C exposure). The UV-C exposure of NDM-1 (*E. coli*) for 1 h and after this experiment was infected with 1 % of NE4 (Coliphage). Cells counting were done 24 h after this procedure. This procedure showed a reduction in the number of cells from 1 x 10^8 to 3 x 10^6 and then decreased a little more to 4 x 10^5 at the end of the processes (1 h in UV-C exposure).

5. Conclusions

This study provided of method for enumeration of ARB and ARG in wastewater treatment to inactivate ARG that infects bacteria and showed the behavior of cells in the UV-C processes. The application of these techniques would improve the enumeration of cells in UV treatment with phage polyvalence NE1 and compare the efficacy versus coliphage NE4 that affects enteric bacteria NDM-1. NE4 was more effective than NE1 in infecting NDM-1. UV irradiation treatment for diminish the risks for human health is an efficient method when associated with coliphage and polyvalence phages for suppression of target bacteria and microbial control.

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