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Optical Decontamination of Large Areas Containing Airborne Microorganisms at Different Phases of Growth

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Abstract: Background: Microorganisms transferred by water, air, and human such as bacteria and viruses can reach inanimate surfaces with high rates of contamination. In general, the objectives of the research were to evaluate the decontamination of large areas such as floors, using a mobile device with ultraviolet C light at different delivery times and also to evaluate these microbial loads in stationary or latent phases. Material and methods: The microbial inactivation effects of this device were measured through the recovery of viable bacteria in different points of the applied area. Results: A significant microbial reduction ($p \leq 0.05$) of 60-87% was obtained in general for all groups and for groups with stationary phase reductions of 100%. Conclusions: microbial inactivation with UV-C ultraviolet light administration rates presents the possibility of potential use on surfaces of large areas for the decontamination of microorganisms in latent and stationary phases.

Keywords: Ultraviolet light, UV-C, decontamination, surface, latent, lag-phase, bacteria.

1. Introduction

The microorganism transmission vehicles can occur mainly from air particles and water droplets, which can be deposited and remain on surfaces such as equipment, floors, walls, and food. The spread of microorganisms can vary significantly between species or even different strains within the same species. Depending on the environmental conditions that are found, create the possibility of forming microbial biofilms that present more excellent stability in these environments even if they present precarious conditions for their development [1, 2].

Cross-contamination is one of the most important vectors in industrial and hospital environments. Besides, it is a quick way for microorganisms to reach humans. An efficient disinfection system is essential to guarantee people's health and in industries, the quality of the final product. Bacteria are present in these environments because they remain alive in the latency phase, needing only a minimum amount of nutrients, water, and environmental factors to resume their replication [3]. During this period, microorganisms may have slow or even lack growth. This phase presents greater tolerance to antimicrobials but no resistance and can thus support the action of harmful substances to microorganisms until it can acquire conditions in which they can return to multiply [4].

For the disinfection of microorganisms contained in surfaces, it is usual to use chemical agents that in some cases due to their resistance are inefficient for microbial control [5, 6]. There is a great variety of antiseptics and disinfectants that it is important to know how to use depending on their degree of toxicity, in addition the effect on the integrity of the material to which it is being applied, safety in humans and the environment. It is also important to know the necessary consumption for a certain area and its cost.

One method to meet this need quickly, easily, safely, economically, and without generating toxic waste is the short-wave ultraviolet light technique,

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converting it into a technology with many advantages for use as an antimicrobial agent [7]. Studies with UV light have proven its antimicrobial efficiency at wavelengths of 254 nm. The mechanism of UV light's action in the death of microorganisms is based on the formation of pyrimidine dimers in DNA, causing structural damage, preventing its multiplication from occurring [3].

For this reason, this study aims to evaluate the decontamination of large areas and also the effect in latent periods of bacterial growth considering different perspectives of the use of UV-C.

2. Material and Methods

2.1 UV-C Light

The UV-C squeegee is a device developed by the Technological Support Laboratory of the São Carlos Institute (USP). It consists of a low-pressure mercury lamp with a 254 nm range made of aluminum with a portable battery of 7 Ah 12V and has a sensor to turn off the light when there is a variation in the height that must be used. The intensity of the lamp has been measured using a *LabMax-TOP* power meter (*Coherent Inc*., Santa Clara, CA, USA) with a sensor suitable for UV-C radiation (LM2-UV). The duration of exposure and distance from the source to the surface of interest corresponded to a dose of 37 mJ/cm^2

2.2 Application of Technique of Disinfection

UV-C device was applied to the surface areas to be decontaminated, varying the same dose of light at speeds 4.5; 9.0; 22.5 ($cm.s^{-1}$), considering that the distance from the surface and the lamp was less than 2 cm and the time constant for 1 minute. The total cumulative dose of UV-C radiation depended on the intensity of the source.

2.3 Microbiological Analysis

Two protocols that considered microorganisms agglomerated in log phase (culture medium) or lag phase environment (deionized water in phosphate buffer, pH 7). The collection was performed by Swab superficially and by collecting reactivating the bacteria using the technique of recovering surviving microorganisms.

The ISO 18593: 2004 gain protocol was followed to evaluate two methods performed to assess the antimicrobial efficiency of the technique. The microorganisms were recovered from the floor surfaces in a microbiology laboratory at the University of São Paulo (USP). The area to be treated, the size of the equipment, three strategic points for the collection and recovery of surface samples were defined. The first methods were with a solution of pH 7, PBS (provides an environment to maintain viable bacteria without providing nutrients for growth and agglomeration in particular pigmented). Each spot sample was collected with a sterile Swab pre-moistened with PBS and placed in 50 mL Falcon tubes, each falcon containing 10 mL of PBS. Subsequently, all samples were homogenized with a magnetic stirrer (brand) and inoculated in Petri dishes and incubated at 37°C for 24 hours and using the method of colony-forming units counted per milliliter (CFU / mL). This protocol performed before and after treatment with a UV-C.

In the second method used, each point collected with a sterile cotton swab pre-moistened with a BHI solution was inserted into 50 mL of the same medium in Falcon type flasks, each containing 10 mL of BHI. The samples were incubated on a table with orbital shaking at 37°C, 150 rpm for 3 h for their metabolic reactivation, and dilutions performed to count the surviving colonies using the UFC/mL method.

2.4 Statistical Analysis

All experiments were repeated three times with a total of n = 18 data and processed in OriginPro software, 2015 (OriginLab Corporation, Northampton, MA, EE. UU). The results obtained from methods 1 (PBS) and 2 (BHI) were analyzed statistically by

Mann-Whitney using the P-value of 0.05, and for the results of reducing UFC (%) the Kruskal-Wallis comparison test was used. To compare all groups at the P-value of 0.05. All data were analyzed statistically by the median and represented in the graphs with their maximum and minimum.

3. Results

The antimicrobial effect of different speeds with one minute of exposure UV-C light for microbiological collections with incubation in the transparent buffer solution was significant compared to the specifics controls groups ($p < 0.05$). For 4.5; 9.0; and 22.5 ($cm.s^{-1}$), an average reduction of approximately 0.6; 0.7; and 0.6, respectively, for all speeds, which had the minimum total log reduction (Fig. 1A).

For results using culture medium (BHI) with an incubation time of 3 hours, where the bacteria were in constant multiplication, the effect of reducing UV-C light was significant ($p < 0.05$) compared to control samples with speeds of 22.5 and 9 ($cm.s^{-1}$) there was 0, 37 and 0.61 of microbial reduction. However, the speed of 4.5 ($cm.s^{-1}$) did not show a significant difference in the sample control ratio, but it also reduced the total as shown in Fig. 1B.

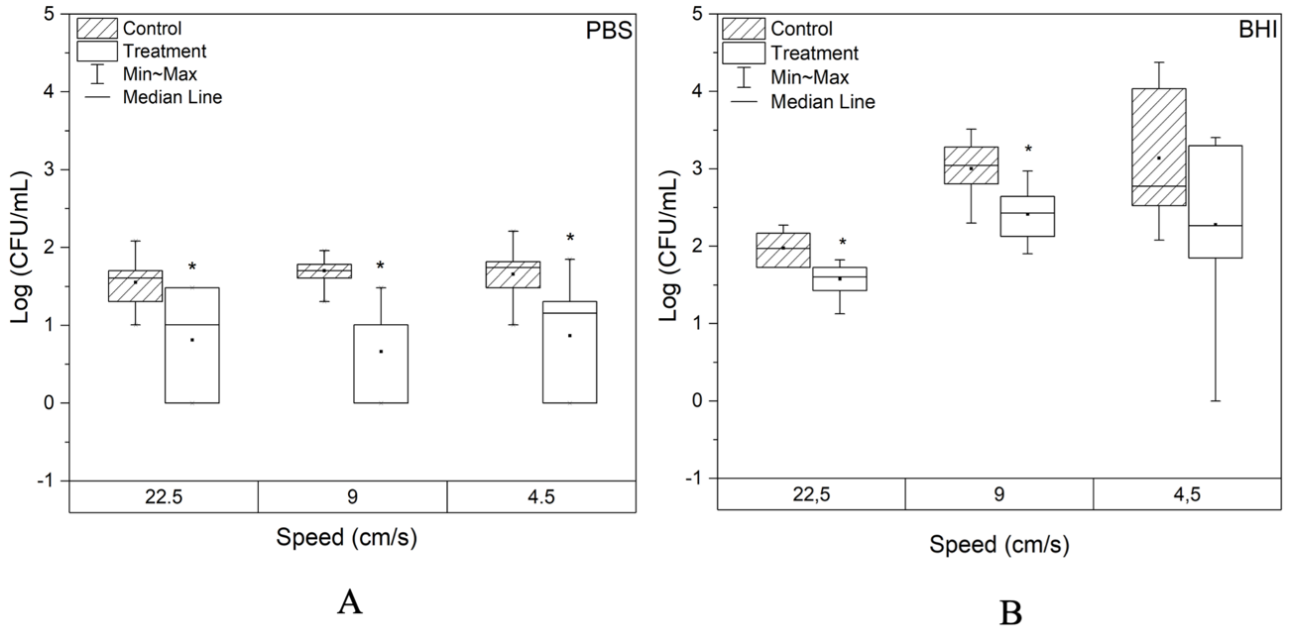

Fig. 1 A) Recovery of log (CFU/mL) before and after exposure to the UV-C lamp for one minute by varying the speed in transparent buffer medium (PBS). B) Recovery of Log (CFU/mL) before and after exposure to the UV-C lamp for one minute by varying the speed in transparent buffer medium culture medium (BHI). The box graph shows the median (median line), the first and third quartiles (outer edges of the box), and the minimum and maximum data; the asterisk indicates the statistical significance of the control ($p \leq 0.05$).

The data required a standardization to evaluate the reductions of viable microorganisms one by one of each sample in their speed, and specific medium studied for this the viable numbers before and after the treatment with UV-C device.

The data presented as a percentage reduction for all experiments (Fig. 2) presented reductions more significant than 60% of viable cells using Kruskal-Wallis statistical analysis.

The use of different cultures did not alter the microbial reduction capacity by the UV-C device ($p > 0.05$); however, the most considerable reduction was obtained with a speed of 9 $cm.s^{-1}$ in a buffer, pH7 of 86.11%, although close reductions achieved in almost all treatments. For treatments using PBS at all speeds, there was a 100% reduction for some experiments.

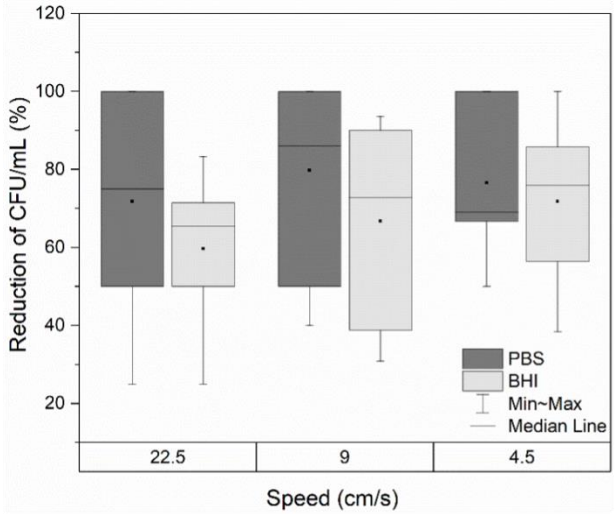

Fig. 2 Percent reduction of bacteria load from an inert surface before exposure one minute to UV-C device at different speeds and solvents. The box chart shows the median (median line), the first and third quartiles (outer edges of box), and the minimum and maximum of the data.

4. Discussion

This study aimed to evaluate the antimicrobial effect of the use of ultraviolet light using a roller-type device capable of moving over large surfaces, facilitating its use of application. UV-C technology was used with the same administration of light doses but varying its delivery through application speeds to surfaces.

The antimicrobial effect was observed both in strains in latent and in stationary phase. This shows that the antimicrobial effect is more significant when is for the latent state. We hypothesize that it is due to the presence of substances that provide an environment rich in nutrients that can alter the color of the environment in which microorganism is located, thus interfering with the penetration of light by the target cells. As shown in Fig. 2, some speeds tested showed a significant difference concerning the sample control, that it did not receive treatment. Optimal conditions of bacterial growth [8] were also considered on the floor surface with are in a latent or stationary state, checking then the effect of UV-C light in different their metabolic phase of grown. This hypothesis shows that if cross-contamination [9], these bacteria can be transferred to nutrient-rich media such as food or humans and may cause disease. For all data in experiments with absence of nutrients (PBS), there was a total microbial reduction for samples and the presence of nutrients [10] (BHI) results only at a speed of 4.5 $cm.s^{1}$; this may be because the recovery of the load was diversified and some bacteria are more susceptible than others, and that also depends on the doses used. The microorganisms irradiated with continuous ultraviolet light can be presented effects of inactivation reversible due to the presence of photochemical repair enzymes, an advantage of using fractional doses is that it does not give DNA cells time to undergo or complete any repair process or adaptation, showing more significant results and avoiding genetic mutations [11].

Corrêa et al. [12], analyzed the effects of ultraviolet light from bacteria collected from different surfaces of a hospital using an ultraviolet light device with a constant dose of 0.78 $J.cm^{2}$, with 80% percent inactivation reductions in most places. However, in this study the light dose studied was 37 $\mu J.cm^{2}$ which showed an average reduction of approximately 60% for different fluences and for de speed 9 $cm.s^{-1}$ in a buffer solution pH7 was 86.11%. We can say that the optical effect may not only be related to the dose delivered to the microorganism but how this dose is delivered, here we relate to the speed. The methods of analysis were also differentiated and here we consider the method of microbial recovery in culture medium to consider changes from the state of latency to an active phase of the microorganisms, and in the study mentioned above the analyzes considered the method of collection by contact that collect and recover bacteria without considering their multiplication capacity.

The variation in speeds did not significantly affect the reduction of bacterial load, this can be explained due to the time of exposure being constant (1 minute) with close speeds, showing that the use of the equipment in a limited area in at least one minute of

UV radiation-C with handling of the equipment, whether in fast, medium or slow movements, is able to decontaminate inert surfaces.

Sneezing, coughing, and contaminated speech droplets can fall on surfaces and remain for several periods causing possible infections [13]. In this study, methodologies with reactivation in mediums rich in nutrients were performed which would simulate these droplets so we can say that this technique used could also inactivate contaminated droplets.

A study using ultraviolet radiation to inactivate SARS-CoV-2 with a light dose of 3.7 mJ/cm^2 was able to reduce 3 Log without viral signs of replication and complete inactivation with 16.9 $mJ.cm^2$ [14]. Giradi *et al.* reported that the use of UV-C irradiation on inanimate surfaces such as plastic, stainless steel and glass which is a physical medium commonly used in hygiene procedures, reduced the SARS-CoV-2 virus by 99.99%, with doses ranging from 10.25 to 23.71 $mJ.cm^2$ [15] .which shows that the doses used are lower than those used in this work, which would also be sufficient to inactivate viral loads such as SARS-CoV-2 using this device with a UV-C light.

By monitoring the changes of the microbial species' death rate, we discover that inhibition to optical stress presented strain intolerance development through an adjustment in the time distribution of light dose in the latency phase of the bacteria, as is not seen in antibiotics [4].

5. Conclusion

The use of ultraviolet light delivered fractionally at different speeds in a small dose was efficient in decontaminating microorganisms and that there was no photo-recovery by the tests performed with incubations of nutrient rich media, showing similar decontamination in the three speeds used, but with better results when higher. Concluding that the use of this form of light supply can be used to decontaminate large areas such as the floor using equipment adapted for UV-C light exposure.

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