

INACTIVATION OF PATHOGENIC BACTERIA USING PULSED UV-LIGHT AND ITS APPLICATION IN WATER DISINFECTION AND QUALITY CONTROL

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Abstract - The lethality of pulsed ultra-violet (UV) rich light for the inactivation of pathogenic bacteria has been investigated. A low pressure xenon filled flash lamps that produced UV intensities have been used. The pulsed operation of the system enable the release of electrical energy stored in the capacitor into the flash lamp within a short time and produces the high current and high peak power required for emitting the intense UV flash. The flash frequency was adjusted to one pulse per second. Several types of bacteria were investigated for their susceptibility to pulsed UV illumination. The treated bacterial populations were reduced and determined by direct viable counts. Among the tested bacteria *Pseudomonas aeruginosa* was the most susceptible to the pulsed UV- light with a 8 log₁₀ cfu/ml reduction after 11 pulses, while the spores of *Bacillus megaterium* was the most resistant and only 4 log₁₀ cfu/ml reduction achieved after 50 pulses of illumination. The results of this study demonstrated that pulsed UV- light technology could be used as an effective method for the inactivation, of pathogenic bacteria in different environments such as drinking water.

Acta Medica Iranica, 44(5): 305-308; 2006

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Key words: UV inactivation, flashlamp, disinfection, bacteria

INTRODUCTION

Chlorine based compounds have, for many years, been widely used as the main disinfectants for drinking water systems due to their high efficiency in eliminating the pathogenic microorganisms found in water. However, some microorganisms, such as the oocysts of *cryptosporidium* and *Giardia* which have been recognized as a threat to the public health are highly resistant to chemical treatments (1, 2).

The formation of additional disinfection by-products (DBP_s) and their toxicological effects, are

also undesirable because of the concerns over their toxicity as potential carcinogens (3, 4). These disadvantages have led to the advancement of alternative antimicrobial technologies such as UV treatment. Ultraviolet processing involves the use of radiation from the ultraviolet region of the electromagnetic spectrum for purposes of disinfection.

The wavelength for UV processing ranges from 200 to 280 nm, called the germicidal range, since it effectively inactivates the microorganisms. The germicidal properties of UV irradiation are mainly due to DNA mutations induced through absorption of UV light by DNA molecules (5, 6).

The aim of this study was to evaluate the effectiveness of pulse UV-light for the inactivation of pathogenic bacteria, and the potential use of such technique in water disinfection.

Received: 25 May 2005, Revised: 27 Jul. 2006, Accepted: 11 Sep. 2006

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MATERIALS AND METHODS

Bacterial Strains

The following bacteria used in this study were obtained from the National Collection of Type Cultures (NCTC), *Escherichia coli* NCTC 9001, *Bacillus cereus* NCTC 11145, *Pseudomonas aeruginosa* NCTC 8203 and *Bacillus megaterium* NCTC 11143.

Preparation of cultures for pulsed UV-light inactivation studies

The test strains were inoculated into 100 ml of Tryptone Soya Yeast Extract broth (Oxoid, UK), and were incubated at 37°C under rotary conditions (150 rev/min) for 18 hours. 30 ml of each sample was suspended into 270 ml of sterile ringer solution (Oxoid, UK). One ml of sample was taken and serial dilution up to 10⁻⁷ were made for the plate count. One ml of each dilution was removed, and enumerated in triplicate on nutrient agar plates to determine the viable count. The agar plates were incubated at 37°C for 20 hours and the number of colonies was counted by the standard plate count technique. Only dilutions which indicate 20 to 200 colonies on each plate were used in quantization.

A volume of 20 ml of the suspended culture was transferred into a standard Petri dish for exposure to the pulsed UV-light treatment. The samples were exposed to the pulsed UV-light for chosen number of pulses. The pulsed flash lamp was located 8 cm above the Petri dish and positioned horizontally. For all experiments, the charging voltage was set to 1

KV, corresponding to an energy per pulse of 20 J. Following exposure by the appropriate number of pulses, treated samples and control samples were enumerated on nutrient agar plates after overnight incubation at 37°C.

Spore selection

Bacillus cereus or *Bacillus megaterium* were grown on by Tryptone Soya Yeast Extract broth (Oxoid, UK) for 2 days at 37°C and then incubated at room temperature for 8 hours to form spores from nutrient exhaustion. To kill vegetative cells, samples were placed in an 82°C water bath until samples reached 82°C. After reaching this target temperature, samples remained in the water bath for 15 additional minutes.

Pulsed Operation

The pulsed UV system is developed by combining power and flash lamp technology. It differs from the traditional continuous UV in that the much higher irradiance of UV illumination can be produced within a short time. The pulsed operation of this system enables the release of electrical energy stored in the capacitor into the flash lamp within a short time and produces the high current and high peak power required for emitting the intense UV flash. The arrangement of pulsed UV system is shown in Figure 1. The lamp box holding the xenon flashlamp is seated in a UV shielding box made of PVC material. The Petri dish holding 20 ml of the tested sample is placed on the bottom of the shielding box, with the centre of the Petri dish

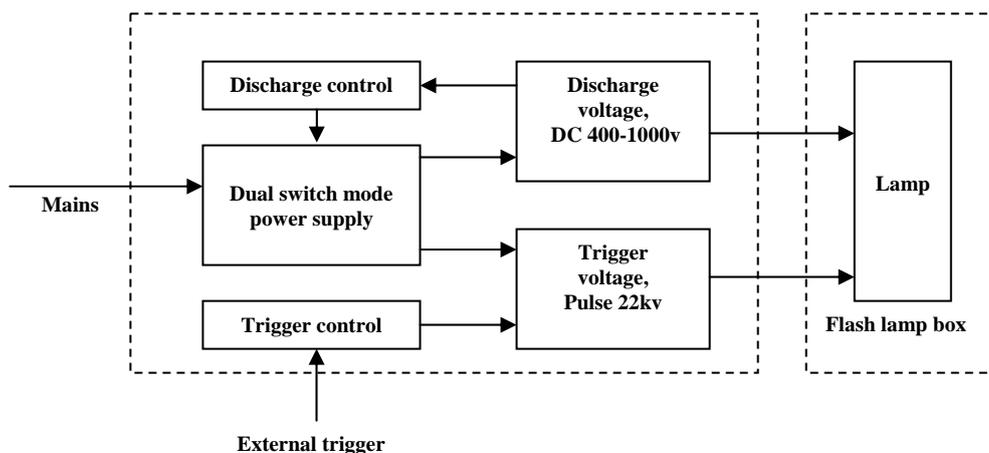


Fig. 1. Block diagram of the pulsed UV system.

aligned vertically with the flash lamp. This configuration gives a distance of 8 cm from flash lamp to the sample surface.

The pulsed UV source was driven at 1 KV and 1 pulse per second throughout the experiments. This allows an electrical energy of 20 J to be dissipated into the flash lamp for each pulse. The flash lamp is operated for 5 min prior to UV exposures to reach a consistent UV output. The dose of UV radiation is controlled by the number of pulses applied.

RESULTS

Inactivation of bacteria

The selected bacteria were inactivated with pulsed UV-light to determine their susceptibility and dose response. The results of pulsed UV-light treatment of the tested bacteria are summarized in figure 2. Of the four tested bacteria *Pseudomonas aeruginosa* was the most susceptible to the pulsed UV-light, with a 8 log₁₀ CFU/ml reduction after 11 pulses, while *Bacillus megaterium* was the most resistant, and only 4 log₁₀ CFU/ml reduction achieved after 50 pulses. With the same treatment 8 and 7 log₁₀ CFU/ml reduction were achieved after 17 and 50 pulses with *Escherichia coli* and *Bacillus cereus* respectively.

The effect of pulsed UV-light on the spores populations of *Bacillus megaterium* and *Bacillus cereus* also were investigated. The results are shown in Figure 3. As it is shown in Figure 3, the spores of *Bacillus megaterium* were the most resistant and only 2 log₁₀ CFU/ml reduction were achieved after 50 pulses, while 5 log₁₀ CFU/ml reduction were achieved after 25 pulses with spores of *Bacillus cereus*.

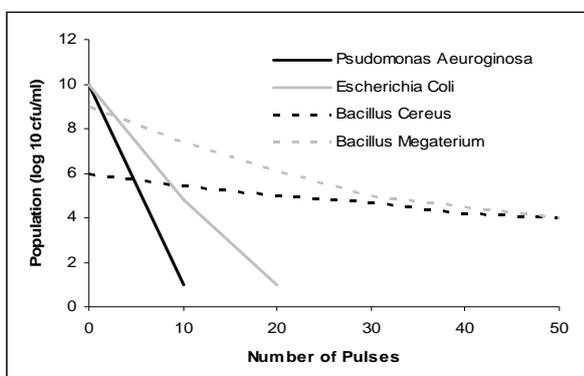


Fig. 2. Inactivation of bacteria with pulsed UV-light.

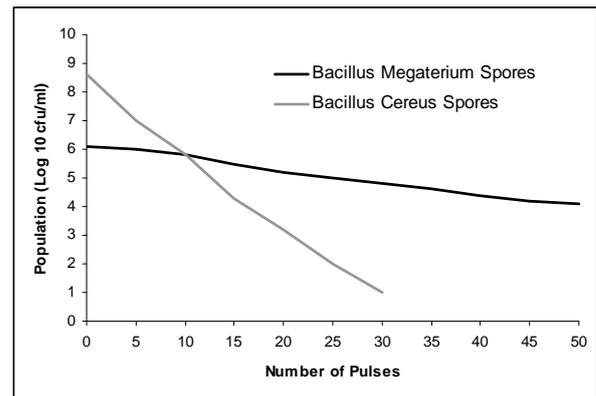


Fig. 3. Effect of pulsed UV-light on *Bacillus megaterium* and *Bacillus cereus* spores.

DISCUSSION

The results obtained here demonstrate the difference in susceptibility of different microorganisms to pulsed UV-light. The Gram-positive bacteria shows higher resistance than Gram-negative bacteria examined. This is in agreement with previous findings (7, 8). It is not clear if this difference in susceptibility to UV radiation is caused by the difference in cell-wall structure between two types of bacteria. The Gram-positive bacteria have many layers of peptidoglycan, forming thick and rigid cell walls, while Gram-negative bacteria have only a single layer or a few layers of peptidoglycan (9). Within the Gram-positive spore forming bacteria, differences in susceptibility were found to exist. *Bacillus megaterium* was found to be more susceptible to pulsed UV than *Bacillus cereus*. This might be due to the size of bacterium, since similar results were obtained when the susceptibility of their spores were compared. The spores of *bacillus megaterium* were found to be more resistant than *Bacillus cereus*.

The results of this study showed the germicidal efficiency of pulsed UV-light, as an alternative method for the inactivation of microorganisms in different environments, such as drinking water.

Conflict of interests

The authors declare that they have no competing interests.

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