Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2013.860627

53 (2015) 1075–1081
January

Taylor & Francis
Taylor & Francis Group

The impact of power supply frequency of a low pressure UV lamp on bacterial viability and activities

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Received 20 May 2013; Accepted 8 October 2013

ABSTRACT

We propose in this study to explore the impact of the power supply frequency and the spectral radiant flux emitted by a germicidal UV lamp on the bacterial viability and activities. Two types of power supplies are used, a traditional electromagnetic ballast taken as a reference and electronic ballast allowing modulating frequencies. To evaluate energy emitted in the UV bandwidth, a photometric system composed of an integrating cylinder, a monochromator, and a CCD camera is mounted. The bacterial viability and activities were repressed when we used a high-frequency UV lamp. Accordingly, the regulation of traditional UV lamp at a high frequency supply can provide an enhancement of UV disinfection process and guarantee the safety of treated water, without any environmental and sanitary hazards.

Keywords: UV irradiation; Low pressure lamp; Radiant flux; Bio-dosimetry; Reactivation; Bacterial virulence factors

1. Introduction

Disinfection, as applied in water and wastewater treatment, is a process by which pathogenic microorganisms are inactivated to provide public health protection. Chlorination has been the dominant method employed for disinfection for almost 100 years; however, it is no longer the disinfection method automatically chosen for either water or wastewater treatment because of potential problems with disinfection by-products and associated toxicity in treated water. Among the alternatives to conventional chlorination, ultraviolet (UV) irradiation is chosen most frequently.

The vast majority of existing UV disinfection systems employs low-pressure mercury arc lamps as their source of radiation. These lamps, which emit roughly 85% of their output power at a wavelength of 253.7 nm, have been shown to be highly effective for inducing photo-biochemical changes in nucleic acids and proteins, without inducing significant photochemical changes in other constituents in aquatic systems [1].

In most water and wastewater applications, UV irradiation accomplishes microbial inactivation without any quantifiable adverse toxicological effects. In addition, UV disinfection is a rapid process; little contact time (on the order of seconds rather than minutes) is required, such that UV equipment

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Presented at the International Joint CB-WR-MED Conference / 2nd AOP'Tunisia Conference for Sustainable Water Management, 24–27 April 2013, Tunis, Tunisia

occupies less space than that necessary for chlorination or ozonation [2].

The effectiveness of UV light in biological inactivation arises primarily from the fact that DNA molecules absorb UV photons between 200 and 300 nm, with peak absorption at 260 nm [3]. This absorption creates damages in the DNA by altering nucleotide base pairing and creating new linkages between adjacent nucleotides on the same DNA strand. This damage occurs particularly between pyrimidine bases.

Two types of mutagenic lesions in DNA were determined; cyclobutane pyrimidine dimers formed between the C–4 and C–5 positions of adjacent thymidine or cytosine residues and pyrimidine (6–4) pyrimidone (6–4) photoproducts formed between the C6 and C4 position of adjacent pyrimidine residues, most often between T–C and C–C residues [3, 4].

If the damage goes unrepaired, the accumulation of DNA photoproducts can be lethal to cells through the blockage of DNA replication and RNA transcription, which ultimately result in reproductive cell death [4].

However, UV disinfection is noted to have some problems, one of them is reactivation. Indeed, biological systems have evolved mechanisms to appropriately respond to environmental stresses that can damage proteins and DNA [5]. In this case, in response to UV damage, bacteria generally possess molecular mechanisms to restore DNA lesions.

The effectiveness of an UV water disinfection device is determined by the UV dose. The UV dose is defined as the product of UV intensity expressed in milliwatts per square centimeter (mW/cm^2) and the exposure time of the fluid or particle to be irradiated expressed in seconds (s). Units commonly used for UV dose are mJ/cm^2 (equivalent to mWs/cm^2) in North America and J/m^2 in Europe [1,6].

The UV dose or UV fluency dependants of the geometry, the surface properties, and the hydrodynamics properties of the reactor. To infer applied UV dose, the bio-dosimetric study can be used.

We propose in this work to study the effectiveness of UVC water disinfection process using two type of electronic power supply of low pressure lamp: classic electronic power frequency of 50 Hz and high electronic frequency ballast 64 kHz. Thereby, to evaluate the efficacy and the impact of each electronic power supply UV lamp a series of bio-assays were realized, such as the study of bacterial inactivation, the exploitation of post irradiated bacterial reactivation, and the control of several virulence factors expressed by a gram-negative environmental species and an opportunistic microorganism, *Pseudomonas aeruginosa*.

2. Methods

2.1. Bacterial strains

The *P. aeruginosa* strain used in this study was obtained from the American Type Culture Collection (ATCC 15,442). Cultures were grown in Luria–Bertani broth (LB; in g/l: 10 tryptone, 5 yeast extract, 10 NaCl) or on LB solidified with 15 g/l agar (LBA). Saline [0.85% (wt/vol) NaCl] was used for cell suspensions during UV irradiation.

2.2. The laboratory UV device

The laboratory UV device was built with the cooperation of the company Guy Daric S.A (Aubervilliers, France). This prototype contained a sliding rack, with an irradiation board which could receive at the same time six Petri dishes 90 mm diameter. A germicidal low-pressure mercury vapour discharge lamp (length = 900 mm, diameter = 13 mm, power of UV emission at 253.7 nm = 55 W) with reflector could be adjusted in height above the irradiation board. The lamp was supplied via electric ballast and the ozone produced in the irradiation room was removed by an extractor.

2.3. Instrumentation for spectral measurements

The implemented experimental set-up performs photometric and radiometric measurements. It consists of an integrating photometric cylinder (radius = 0.7 m, length = 1.8 m), a monochromator (120 mm focal length, 1,200 grooves mm^{-1}) with input optics and detector system, a Hagner digital lux meter (F2X), a CCD camera and an automated system controlled by a personal computer.

2.4. Viable cell counts

Viable cell counts were taken before and immediately after UV exposure. A 100 µl portion of each irradiated samples was removed in order to prepare serial dilutions in PBS buffer. A volume equal to 100 µl of the appropriate serial dilutions was spread in duplicate onto LB agar. The number of colony-forming unit (CFU/ml) or a number of viable and cultivable bacteria was determined after 24 h of incubation at 37˚C. The fraction of viable and cultivable bacteria was calculated by dividing the number of CFU in the UV-treated sample (N) by the number of CFU determined at time zero before UV irradiation (N_0).

2.5. Bacterial reactivation in darkness or visible light condition

UV-irradiated samples were divided and transferred into two separate sterile Petri dishes. One of the two Petri dishes was exposed to visible light to examine potential photo repair and one was covered with foil to allow for potential dark repair at room temperature.

2.6. Swarming motility

Swarming motility was assayed on 0.5% agar LB plates supplemented with 0.4% glucose [7]. Swarming plates were incubated overnight at 30°C, and placed at room temperature for an additional 24–48 h prior to measurement of the swarming diameter. Swarming assays were repeated in triplicate.

2.7. Biofilm formation by P. aeruginosa

Biofilm phage susceptibility was obtained by study of phage infectivity into their free-cells or planktonic form and with biofilm community.

Biofilm formation was quantified as described [8]. Before UVC irradiation, an overnight culture of *P. aeruginosa* was diluted 100-fold in fresh TSB broth and 200 µl was added to each well of a 96 wells of Microtiter plate. Cells were grown for 18 h at 30°C (preformed biofilm) before they were stained with crystal violet and quantified.

3. Results

3.1. The inactivation kinetic: UV dose-response

In this study, we used *P. aeruginosa* ATCC15422, as a representative bacteria to monitor the impact of the power supply frequency and the spectral radiant flux emitted by a germicidal UV lamp on the bacterial viability and activities.

P. aeruginosa is an ubiquitous bacteria recognized by its genetic and metabolic flexibility. This bacterial strain is well studied in terms of UV dose/response relationship, including its process of different mechanisms of DNA repair (photo repair and dark repair). In addition, this strain of bacteria can express many cell-associated and extracellular virulence factors. There virulence factors play an important pathological role in the colonization, the survival of the bacteria, and the invasion of tissues.

Fig. 1 shows the inactivation kinetic of *P. aeruginosa* for two electronic power supply of UVC low pressure lamp: traditional power supply at 50 Hz (F1) and high frequency power supply at 64 kHz (F2).

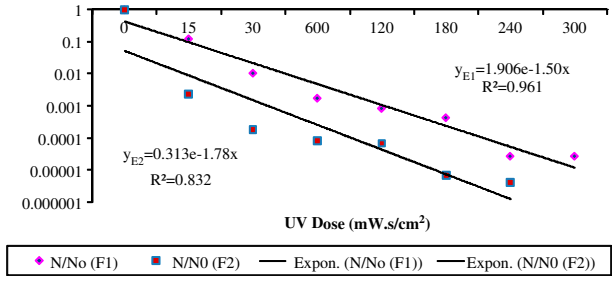


Fig. 1. Inactivation kinetic of *P. aeruginosa* strain using two electronic power supply of the same low pressure lamp: traditional power supply at 50 Hz (F1) and high frequency power supply at 64 kHz (F2).

Several mathematical relationships have been developed to describe bacterial responses to UV irradiation. In this study, the inactivation kinetics was simulated by a mathematical model called *Series-Events* model with modification [9].

$$\frac{N}{N_0} = A\exp(-K \times D_{uv}) \sum_{i=0}^{n-1} \frac{(K \times D_{uv})^i}{i!} \quad (1)$$

where N_0: is the number of viable cultivable bacteria before exposure to UV light, N: is the number of viable cultivable bacteria after exposure to UV light at time t, A: is the retention of viability rate following UV irradiation, K: is the coefficient of lethality, D: UVC dose expressed by mJ.cm^{-2}, and n: is the threshold level of series-event model. The constants K and A were determined by linear regression.

This kinetic model can establish the relationship between a physical parameter and the biological one (dose/response) and determine the kinetic parameters needed to compare the effectiveness of two type of electronic power supply lamp on bacterial inactivation.

The lethal coefficient (K) and the retention of viability rate following UVC irradiation (A) for both electronic power supply of the same low pressure lamp: traditional power at 50 Hz (F1) and high frequency power at 64 kHz (F2).

Fig. 1 shows the increase of bacterial reduction (N/N_0) correlated with the increase of exposure time (s). This bacterial reduction is probably due to the DNA photo damages accumulation induced by germicidal UV light emitted by UVC lamp [2,3].

The same curve (Fig. 1) shows a difference in the level of the inactivation kinetic for the both studied electronic power supply. According to Hassen et al. [10], the greater the coefficient K, the lower is the bacterial tolerance to UV. Based on this argument, we can deduce the effectiveness of high frequency power

supply of germicidal lamp (F2) compared to the traditional ballast lamp (F1). Indeed, K is equal to 1.5 when we used traditional ballast (F1). This coefficient of lethality is equal to 1.78 for the second power supply UVC lamp (F2).

The analysis of the second kinetic parameter (A) determined by *Series-Events* model for both high frequency and traditional power supply of low pressure lamp shows that the irradiated bacteria with high frequency electronic power supply (A = 0.31) lost the viability and the cultivability faster than bacteria irradiated with traditional ballast (A = 1.906).

In addition, we can deduce that the exposure time required to inactivating 99.99% (4 U-$\log_{10}$) of viable and cultivable bacteria decreases by two times when we use a UV lamp powered at 64 kHz.

The decrease of the exposure time was probably due to the increase of UV radiant flux emitted by a high-frequency UVC lamp. This probability is checked by measurement of the line spectral flux 253.7 nm using a spectroradiometer (Fig. 2).

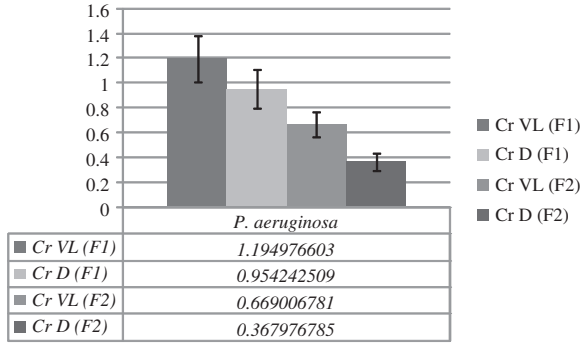
3.2. Bacterial reactivation

Fig. 3 shows reactivation of post-irradiated *P. aeruginosa* after a rest time in visible light and in darkness, respectively.

To semi-quantify the reactivation level of post-irradiated bacteria in visible light and/or in darkness, a log ratio was determined according to a modified version of the Lindauer and Darb equation (1994) [11]:

$$C_r = \mathrm{Log}\, Nr/N_{\mathrm{UV}} \quad (2)$$

where Cr: is the coefficient of reactivation; N_r = Number of viable and cultivable bacteria after a rest time in the visible light or/and in the darkness; UV radiation and N_{UV}: number of viable and cultivable bacteria after UV radiation.



	P. aeruginosa
Cr VL (F1)	*1.194976603*
Cr D (F1)	*0.954242509*
Cr VL (F2)	*0.669006781*
Cr D (F2)	*0.367976785*

Fig. 3. Determination of bacterial reactivation factor (Cr) for *P. aeruginosa* ATCC 15,422 after a rest time in presence of visible light (VL) and in the darkness (D) for two electronic power supply low pressure UVC lamp at 50 Hz (F1) and at 64 kHz (F2).

According to Lindauer and Darby (1994), the reported log value ranges from 1 to 3.4. When the C_r is < 1, there is no reactivation or no UV-DNA damage repair; when C_r range between 1 and 3.4, we can conclude that reactivation occurs in the darkness or/and in visible light; when C_r is > 3.4, there are no UV effects and the cells grow naturally without any environmental stress.

Fig. 3 shows for the traditional ballast, post-irradiated *P. aeruginosa* was reactivated after a rest time in visible light (C_r = 1.194) than in the darkness (C_r = 0.95). However, when we tested the modified power supply lamp supplied at 64 kHz, the coefficients of reactivation (Cr) of tested bacteria after a rest time in the visible light (C_r = 0.669) and in the darkness (C_r = 0.367) were inferior to 1. Thus, when

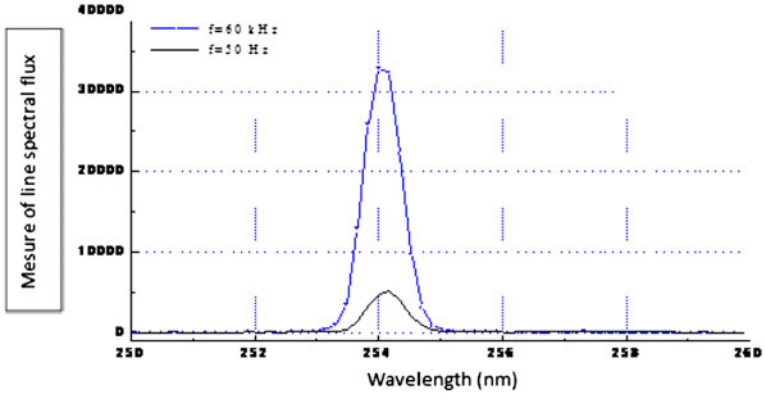


Fig. 2. Spectral energetic flux of the 254 nm line at 50 Hz (F1) and at 64 kHz (F2).

we use high frequency UV lamp, we can prevent the resuscitation of post-treated bacteria and subsequently, we can provide an effective water disinfection step.

3.3. Biofilm formation and swarming motility vs. power supply UV lamp

The aim of this part of the study was to monitor the expression of multicellular behavior: biofilm formation and swarming motility; under two type of electronic power supply low pressure UV lamp: traditional ballast (F1) and high-frequency power supply low-pressure UV lamp (F2).

P. aeruginosa is an opportunistic pathogen that commonly causes infection in immune-compromised individuals. This bacterium forms complex communities known as biofilms. Biofilms are also beneficial for bioremediation processes in terms of eliminating oil spills and sewage treatment; however, they can be harmful, particularly, in the food industry sector and in water distribution systems.

Due to its complexity, the formation of biofilms is regulated at different stages via diverse mechanisms [12]. The most studied regulatory mechanism that has been found to control the production of EPS, biofilm formation, and differentiation is quorum sensing (QS) regulation system [13]. QS allows bacteria to maintain cell-cell communication and also regulate the expression of specific genes in response to changes in cell population density [12–14]. In general, the QS process involves the production, release and detection of chemical signaling molecules, thus allowing microbial cells to regulate gene expression in a cell-density-dependent manner [15]. At a given population density, the genes involved in biofilm differentiation and maturation are activated [16]. Consequently, by the investigation of biofilm formation we can explore the impact of different phases of bacterial regulation and virulence factors expression vs. electronic power supply lamp.

Additionally, we were investigating the retention of bacterial mobility after UV irradiation for both power supply frequency. The bacterium *P. aeruginosa* is capable of three types of motilities: swimming, twitching, and swarming. The latter is characterized by a fast and coordinated group movement over a semi-solid surface resulting from intercellular interactions and morphological differentiation. A striking feature of swarming motility is the complex fractal-like patterns displayed by migrating bacteria, while they move away from their inoculation point. This type of group behavior is still poorly understood and its characterization provides important information on bacterial structured communities such as biofilms [17].

P. aeruginosa has an intrinsic sessile formation behavior that leads to biofilms. Nonetheless, biofilm formation shares many features with swarming motility. Indeed, both are community behaviors that bacteria exhibit on surfaces. Moreover, common sets of bacterial surface structures (flagella, tfp, EPS) and secreted products (rhamnolipids) influence both biofilm and swarming behaviors, and common nutritional cues affect the extent and structure of both swarming motility and biofilms production [16].

Therefore, to highlight the performance of high frequency power supply lamp at 64 kHz UV lamp, we have explored community behavior of *P. aeruginosa*: swarming motility and biofilm production; after UV exposure time allowing the inactivation of 99.99% of viable and cultivable bacteria irradiated by a germicidal lamp supplied at 64 kHz; thus, after irradiation time equal to 120 s (Fig. 4). The exposure time was determined through the inactivation kinetic of *P. aeruginosa* ATCC 15,422 (Fig. 1).

Figs. 4 and 5 show the decrease of biofilm formation and swarming motility after an exposure time equal to 120 s. The decrease of virulence factors expressed by a biological model after UV irradiation by a high frequency powered UVC lamp can be explained by the fact that the bacteria have received a lethal UVC dose, reducing bacterial sustainability by accumulation of photoproducts surpassing the capability of bacterial DNA repair mechanisms and consequently leading to a decrease in virulence factor expression. In addition, in bacteria, the regulation of many important changes in gene expression is mediated by systems of signaling between cells known as QS [13]. Indeed, to withstand the hostile conditions (UV radiation, starvation, etc), microorganism will sense their density and number through the presence of signals that diffuse freely across cell membranes

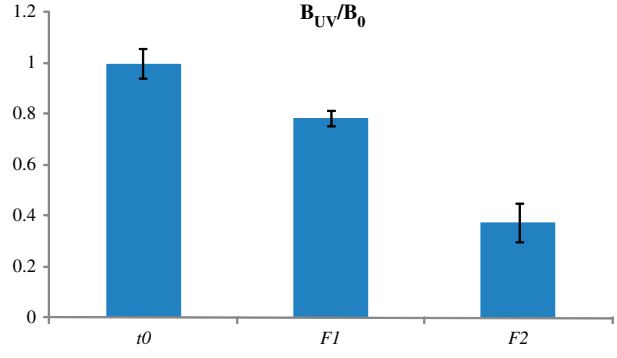


Fig. 4. Biofilm production by *P. aeruginosa* before UV radiation at time zero t_0 and after irradiation by two electronic power supply UVC lamp at 50 Hz (F1) and at 64 kHz (F2).

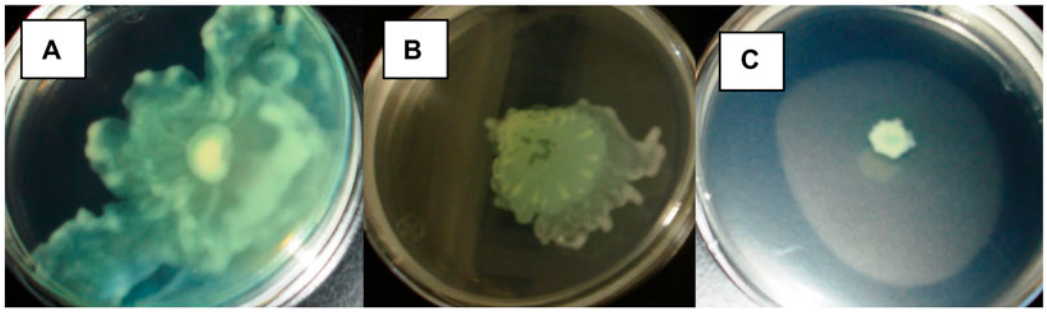


Fig. 5. Swarming motility on LB plates of *P. aeruginosa* before UV radiation at time zero [A] and after irradiation by two electronic power supply UVC lamp at 50 Hz (F1) [B] and at 64 kHz (F2) [C].

and between cells. via auto-induced positive feedback mechanism, a population of cells can quickly induce the appropriate phenotypes required to respond to a particular environmental condition or to proceed with the differentiation process of the population [18].

This repression of bacterial community behavior was enhanced when we irradiate the tested bacteria by a high frequency powered UVC lamp (F2). Indeed, the biofilm production was repressed nearly two times compared to the biofilm amount determined after UV irradiation by a traditional lamp (F1) as well as for the swarming motility. The diameter of Swarm motility assays for *P. aeruginosa* (Fig. 5) shows a reduction of the coordinated group movement over a semi-solid surface. This motility reduction was improved after the use of high frequency power supply lamp at 64 kHz. Indeed, the enrichment of UV germicidal rays (Fig. 2), allows at shorter time, the decrease of bacterial viability, the reduction of bacterial resuscitation and the repression the expression of virulence factors.

4. Conclusion

After a series of bio-assays, we can conclude that the use of high frequency power supply lamp at 64 kHz have several advantages. We can include these benefits: (i) the maintain of a monochromatic radiation at 253.7 nm; (ii) the increase of emitted UVC intensity by low pressure UV lamp; (iii) the UV exposure time is shorter than for the traditional ballast, this saving time can be used to increase the water flow in case of application for industrial scale; (iv) the reduction of sanitary risks related to the bacterial reactivation and expression of virulence factors after disinfection by UVC irradiation; and (v) the optimization and the enhancement of UVC disinfection systems using high frequency power supply lamp at 64 kHz.

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