Desalination 285 (2012) 219–225

Contents lists available at SciVerse ScienceDirect

Desalination

journal homepage: www.elsevier.com/locate/desal

# Multivariate optimization of fecal bioindicator inactivation by coupling UV-A and UV-C LEDs

A.-C. Chevremont [a,b,*], A.-M. Farnet [b], M. Sergent [c], B. Coulomb [a], J.-L. Boudenne [a]

[a] Université de Provence-CNRS, FR ECCOREV, Laboratoire Chimie Provence (UMR6264), Equipe «Chimie de l'Environnement Continental», 3 place Victor Hugo, case 29, 13331 Marseille cedex 3, France
[b] Université Paul Cézanne-CNRS, FR ECCOREV, Institut Méditerranéen d'Ecologie et de Paléoécologie (UMR6116), Equipe «Ecologie Microbienne et Biotechnologies», Avenue Escadrille Normandie-Niemen, Boîte 452, 13397 Marseille cedex 20, France
[c] Université Paul Cézanne-CNRS, FR ECCOREV, Institut des Sciences Moléculaires de Marseille (UMR6263), Equipe «Systèmes Chimiques Complexes», Avenue Escadrille Normandie-Niemen, Boîte 512, 13397 Marseille cedex 20, France

ARTICLE INFO

*Article history:*
Received 24 June 2011
Received in revised form 29 September 2011
Accepted 6 October 2011
Available online 12 November 2011

*Keywords:*
Bacteria inactivation
Coupled wavelength
Ultraviolet light-emitting diodes (UV-LED)
Water recycling

ABSTRACT

The development of new technologies for water recycling is a priority for arid and semi-arid countries such as those of the Mediterranean basin. The aim of this study was to test the efficiency of UV-A and UV-C light emitting diodes (UV-LEDs) on bacteria inactivation. We used *Escherichia coli* and *Enterococcus faecalis*, bioindicators of fecal pollution typically found in urban wastewaters. An experimental design was performed to discriminate weight of factors influencing bacteria inactivation yields and reactivation phenomena. Four parameters were tested on simple bacterial cultures: pH, bacterial density, exposure time and wavelength. It appears that the exposure time and wavelength used have a significant effect on the response. The 280/365 nm or 280/405 nm coupled wavelengths, have the most important bactericidal effect, and we also note the absence of bacterial reactivation after 60 s of exposure to UV.



## 1. Introduction

Continuous population growth and economic development increase the water demand and encourage governments to look for alternative water sources, such as reuse and recycling of wastewaters, especially for irrigation purposes [5, 23, 24]. Moreover, implementation of more stringent regulations about quality of wastewaters discharged into the aquatic environment (as the European Directive "concerning the management of bathing water quality", 2006) implies design of more sophisticated or refining treatments. Consequently, developing new technologies for recycling wastewaters or treating raw waters is a priority for many countries, especially in relation to microbiological criteria.

Use of ultraviolet (UV) light has become an alternative to chlorine and other chemical disinfectants and has proven to be an efficient technique to eliminate many potentially pathogenic microorganisms, even in case of turbid waters [12, 13, 15, 18]. Indeed, UV radiations are known to cause damage on DNA, preventing replication, transcription and thus, indirectly, translation.

However, many microorganisms can repair DNA damages caused by UV radiation via enzymatic reactions. Two different mechanisms are distinguished depending on light availability: photoreactivation and dark-repair [19, 20, 25]. Photoreactivation is a natural process in which bacterial cells can partially recover from ultraviolet damage when visible and UV wavelengths of light reverse DNA damage by monomerizing cyclobutane pyrimidine dimers. In this case, repair is due to an enzyme called photolyase. In dark repair the damage is reversed by the action of a number of different enzymes. All of these enzymes must initially be activated by an energy source, which may be visible light (300–500 nm) or nutrients that exist within the cell. [16] suggests that the enzymatic repair mechanism requires at least two enzyme systems: an exonuclease systems (i.e. to disrupt the thymine–thymine linkage), and a polymerase system to reinsert the thymine bases on the adenosine sites of the complementary strain of DNA.

The majority of UV disinfection systems use low or medium-pressure mercury lamps, first ones emitting predominantly monochromatic UV radiation at a wavelength of 254 nm and second ones emitting polychromatic UV radiation over the wavelength range from 200 to 400 nm. However, mercury vapor lamps have many disadvantages: large size, low resistance to shock and the energy required to operate. Furthermore, these lamps have a short lifespan of approximately 4000–10,000 h and contain mercury, a major environmental contaminant. On the other hand, the use of LEDs to produce UV radiations has many advantages. LEDs are very compact, shock-resistant, do not require much energy to operate and their lifetime exceeds 100,000 h [6]. In addition, LEDs do not contain toxic substances or pollutants: the materials typically used are gallium aluminum nitride (AlGaN) and aluminum nitride (AlN), which are not toxic [14]. Furthermore,

* Corresponding author. Tel.: +33 491288528; fax: +33 491288190.
*E-mail address:* anne.chevremont@live.fr (A.-C. Chevremont).

0011-9164/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.desal.2011.10.006

LEDs use electricity more efficiently, producing little heat. Last but not least, LEDs present the advantage upon lamps to be compact light sources and to produce UV at a one single wavelength, allowing thus to design UV disinfection systems with optimized numbers and wavelengths LED as a function of microorganisms to be inactivated.

Few studies have been conducted so far on water disinfection by UV-LED technology: [17] have tested the efficiency of commercially available UV-LEDs emitting at 365 nm and at 405 nm, [9] have tested high-UV LED emitting at 365 nm and [26] have shown that UV-LED irradiation was efficient to reduce *Escherichia coli* concentration using 269 nm. Until now, these studies have not been conducted with the objective to optimize choice of wavelengths, numbers of LEDs or even time of irradiation.

This preliminary study aims to develop a UV-LED-based protocol under in vitro conditions, by testing the effect of different factors on UV disinfection efficiency. We combine different parameters: bacterial density ($10^5$ or $10^7$ cfu/mL), pH (6 and 8), UV exposure time (10, 20, 30, 60, 120 and 180 s), and wavelengths (254, 365, 280, 405 nm). Wavelengths 254 and 280 nm (UV-C) are potentially the most efficient to eliminate bacteria since they are close to the DNA maximum absorption rate and are responsible for the formation of cyclobutane pyrimidine dimers [9]. Wavelengths 365 and 405 nm are included in the UV-A range which is known to induce the formation of active substances having lethal effects [22], i.e. leading to the formation of oxidative DNA damage [9]. All these wavelengths were tested independently and also coupled (UV-C/UV-A) as follows: 254/365 nm; 280/405 nm and 280/365 nm. The study was performed according to an experimental design. The effectiveness of UV treatment was tested on *E. coli* and *Enterococcus faecalis*, fecal indicator bacteria commonly found in wastewater effluents. The bacterial reduction must reach at least 7 log to be considered as an efficient result in wastewater treatment [27]. To highlight potential different responses within the same species and between species, three strains of *E. coli* (American Type Culture Collection (ATCC) 11303 and ATCC 15597 [4] and Collection of Institut Pasteur (CIP) 6224) and two strains of *E. faecalis* (ATCC 19433 and ATCC 33186) were used. Moreover, specific attention was focused on the reactivation ability of selected microorganisms.

## 2. Materials and methods

### 2.1. Bacterial strains and growth conditions

To test the inter-specific variability, three strains of *E. coli* (ATCC 11303, ATCC 15597 (LGC Standards) and CIP 6224) and two strains of *E. faecalis* (ATCC 19433 and ATCC 33186) were cultivated for 3 h (exponential growth phase) on a nutrient broth medium. Cultures were centrifuged for 15 min at 10,000 rpm (Sorvall®, Evolution RC) and pellets were resuspended in either pH6 phosphate buffer or in pH8 tris(hydroxymethyl)aminomethane buffer. Their optical density (600 nm) was adjusted to 0.100 for *E. coli* and 0.050 for *E. faecalis* ($10^7$ cfu/mL) using the appropriate buffer for dilution. Buffered solutions containing $10^5$ cfu/mL were obtained from dilutions of $10^7$ cfu/mL solutions.

### 2.2. Experimental setup

Four LEDs (manufactured by Seoul Optodevice Co., Ltd., Korea) emitting at 255, 280, 365 or 405 nm respectively were used throughout experiments. These LEDs could be used together or separately and the electronic circuit was connected to a power supply (TTI EL302R Power Supply).

These four LEDs were used with their maximal amperage, as presented in Table 1, along with the other physical properties of these LEDs. Distance between the LEDs and the bacterial cultures was set at 1 cm (according to standard ÖNORM M5873-1). A scheme of the experimental device is shown in Fig. 1. Bacterial cultures (10 mL) were placed in Petri dishes (55 mm diameter) and exposed to each wavelength or wavelengths coupling and for each exposure time. Cultures were homogenized using a magnetic stirrer during exposure.

**Table 1**
Optical and physical properties of UV-LED used during inactivation experiments (data provided by LED suppliers).

| Peak wavelength (nm) | Packaging | Optical power (mW) | Viewing angle 2θ 1/2 (degree) | Maximum current intensity (mA) |
|---|---|---|---|---|
| 255 | TO-39 | 0.125 | 120 | 115 |
| 280 | TO-39 | 0.55 | 120 | 77 |
| 365 | P8 | 350 | 110 | 22 |
| 405 | P8 | 210 | 130 | 46 |

### 2.3. Enumeration of bacteria

After irradiation by the UV-LED system, 1 mL of each bacterial suspension was diluted as necessary with a factor of ten and plated on Tryptone Soy Agar (TSA) medium. Cultures were incubated for 24 h at 37 °C and bacteria were then counted. Irradiated bacterial solutions were kept for 20 h at room temperature and in normal room lighting, and then diluted as necessary and plated on TSA medium to assess bacteria reactivation.

### 2.4. Experimental design and data analysis

As previously mentioned, different parameters are potentially influential on UV disinfection efficiency. On microorganisms, the various factors studied with the tested values were respectively $10^5$ and $10^7$ cfu/mL for the density of microorganisms; 6 and 8 for pH; 60, 120 and 180 s as exposure time; and 254, 280, 365, 405, 254/365, 280/365 and 280/405 nm as tested wavelengths.

In a first step, a screening study has been performed in order to select factors that are probably active and to get a preliminary idea as to their effects. Instead of a traditional "one factor at a time" approach, the experiments were carried out using an experimental design [3] for setting up experiments in such a manner that the required information was obtained as efficiently and precisely as possible.

A screening study allows the direct comparison of two or more values and the postulated mathematical model is simply additive. The reduced reference state model used in a screening design for 2 variables with 2 levels, 1 variable with 3 levels and 1 variable with 7 levels is the following:

$$\text{Disappearance}\% = a_0 + a_1X_{1A} + b_1X_{2A} + g_1X_{3A} + g_2X_{3B} + d_1X_{4A} + d_2X_{4B} + d_3X_{4C} + d_4X_{4D} + d_5X_{4E} + b_6X_{4F}$$

with $X_i = 0$ or 1 (presence–absence variables) in function of the level present for the four factors.

The coefficients $\alpha_1,..\beta_1, \gamma_1, \gamma_2, ..$ define the differential effect on the disappearance percentage of replacing a level (considering as reference state) by another level.

To optimize coefficients estimation of the model, an optimal design of experiments [1, 7] has been chosen: this asymmetrical design presents 14 experiments described in Table 2.

## 3. Results and discussion

The aim of this study was to develop the most effective UV LED protocol as a purification system and also to highlight different degrees in sensitivity to UV within a species and between species. Here we examined the effect of different UV wavelengths emitted by LEDs on three different strains of *E. coli* and two strains of *E. faecalis*. The ATCC 11303, ATCC 15597 (used in other experiments to test the effects of