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PROTOTYPE UNITS TO ADDRESS EMERGING CONTAMINANTS AND E. COLI IN WASTEWATER VIA PHOTODYNAMIC INACTIVATION

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Abstract. *The inappropriate disposal of organic pollutants has been drawing attention from the research community in recent years. One of its consequences is the rise of the emerging contaminants (ECs): a vast array of synthetic products that have the general characteristics of being bioacummulative, not regulated and potentially lead to antimicrobial resistance when found in wastewater. In general, emerging contaminants are endocrine disrupters and include pharmaceuticals and personal care products (PPCPs), artificial sweeteners, hormones, pesticides, illicit drugs, among others. A feasible technique to treat the wastewater contaminated with ECs is the photodynamic inactivation (PDI), which consists in the interaction between a photosensitizer (PS) with a light source of a specific wavelength in order to yield reactive oxygen species (ROS) which in turn will break the molecules of the contaminants. This work is aimed at carrying out laboratory tests with Escherichia coli, a bacterium responsible for various diseases, which will guide the setup definition for the development of prototype treatment units. They are to operate under optimal conditions for the inactivation of selected microorganisms and ECs. The significance of the flow conditions, light irradiance, and PS concentration are on the scope of the future designed experiments.*

Keywords: *emerging contaminants, photodynamic inactivation, wastewater treatment, prototype, antimicrobial photodynamic therapy.*

1. INTRODUCTION

Synthetic products have made life in modern society easier, providing comfort and quickness in health problem solving as well as enhancing productivity in agriculture, to name a few. From an ample list of them there are pharmaceuticals and personal care products (PPCPs), pesticides, hormones, artificial sweeteners and flame retardants. Concern about these contaminants owes to their bioaccumulation, their potential to increase antimicrobial resistance,

the fact that they are not regulated, and also because a significant part of them are endocrine disrupting chemicals (EDCs), being able to alter the normal functioning of organisms, especially wildlife species (Gabarrón, *et al*., 2016), such as causing male fish to lay eggs due to feminine hormone 17β-estradiol (Pereira, *et al*., 2015).

The inappropriate disposal of these chemicals allied to the regular wastewater treatment (WWT) inability to properly degrade them (Pereira, *et al*., 2015) urges new studies to assess this matter, seeking alternatives to their treatment.

A promising way to promote the degradation of the ECs is via the photodynamic inactivation (PDI). This technique is based on the interaction of a photosensitizer (PS), which is a chemical compound that absorbs energy on a certain wavelength, and a light source with that respective wavelength. Once this interaction is achieved, reactive oxygen species (ROS) are generated, free radicals that are able to break down those molecules and even cell structures, potentially destroying pathogenic micro-organisms (Alves, *et al*., 2014). The PDI mechanism is also described in the health sciences as photodynamic therapy (PDT) (Castano, *et al.*, 2005), and when dealing with microbes, the special term antimicrobial photodynamic therapy (aPDT) is generally used.

The main goals of this work are to conduct aPDT *in vitro* tests with *Escherichia coli*, a harmful bacterium, that will help develop prototype units for microorganisms' reduction and the degradation of ECs. We hope this will contribute to establish the optimal conditions under which the prototypes operate according to the PDI method, taking into account the flow rate, light irradiance, and PS concentration. At this moment, the prototypes are on the final stages of their construction.

2. METHODOLOGY

Two prototype units are under development to evaluate the efficiency of the PDI in different configurations. Figure 1 illustrates the lamp and LED designs for the units.

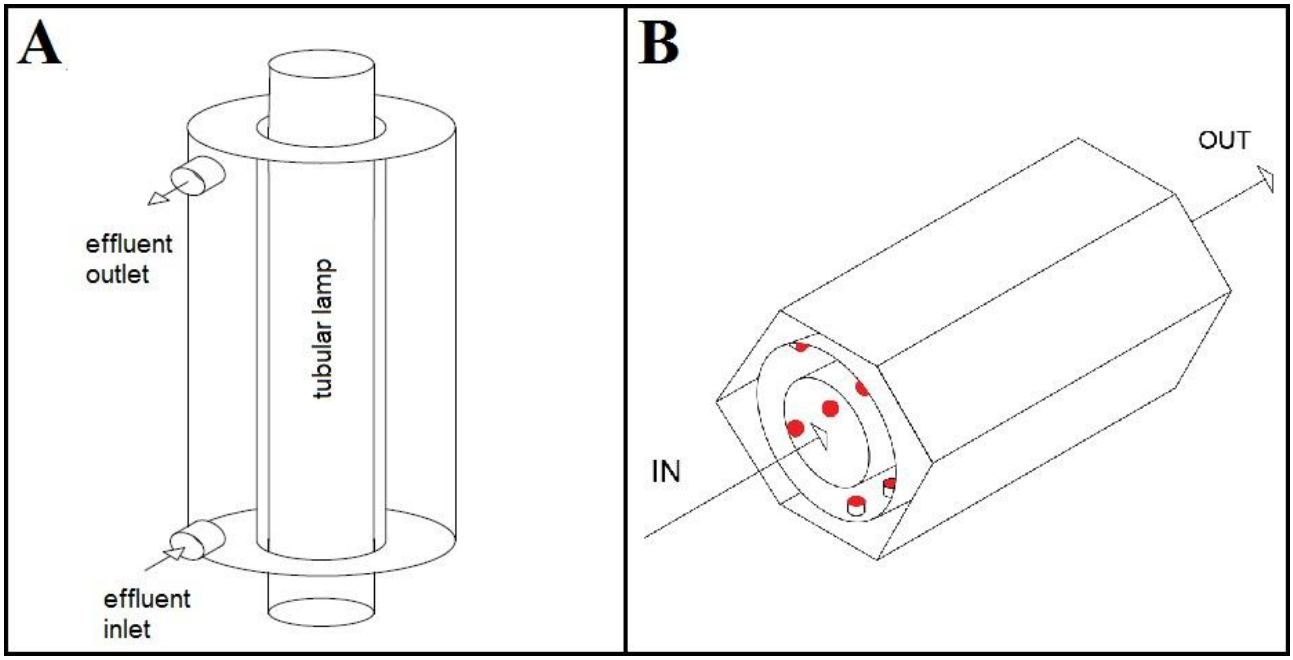

Figure 1. Design of the prototypes. (A) Lamp unit; (B) LED unit.

The main parameters for the analyses are the time of light exposure or residence time, which will be adjusted by the flow rate, PS initial concentration, EC concentration reduction and light irradiance (Wm^{-2}). A peristaltic pump will be used to ensure low flow rates can be achieved. In the lamp design the inlet flow from the bottom will allow an acceptable residence time with a low flow rate.

The PS, or dye, chosen for this project is the methylene blue, which has peaks of absorption at 630 nm and 668 nm (Figure 2). Taking this into account, red light sources are necessary. The LEDs and the tubular lamp wavelength emissions range between 620-650 nm.

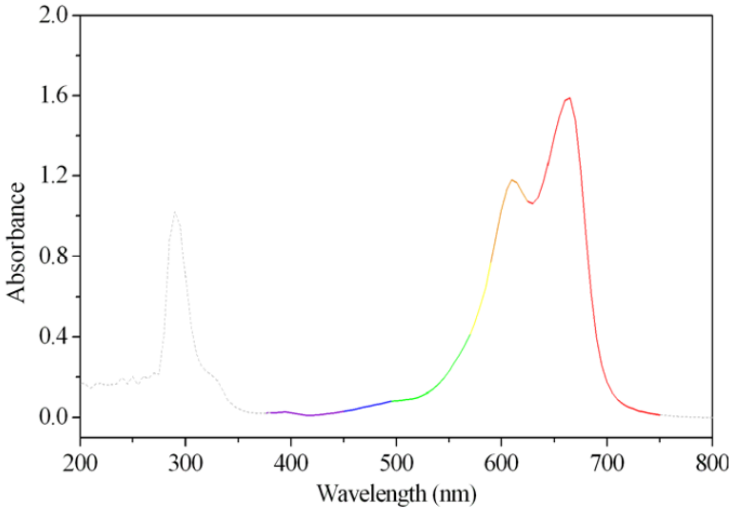

Figure 2. UV-Vis spectrum of methylene blue (Whang, *et al*., 2009).

2.1 *E. coli* Assessment

An aPDT assay was performed in laboratory conditions in order to guide the setup for the tests using the prototypes. Enterotoxic *Escherichia coli* (ETEC) 9 was the microorganism chosen for being a usual pathogen in water quality regulation. It was cultivated in tryp soy agar (TSA) for 24h, at 37°C in an aerobic environment. A concentration of 10^6 colony-forming units (CFU)/mL was attained. It was subject to the treatments on a black plate (Figure 3) and later plated to Petri plates with a concentration of 10^3 CFU/mL. This dilution is necessary so as to guarantee a clear counting of the colonies; otherwise the colonies could be too close to one another, hindering the process of unit counting.

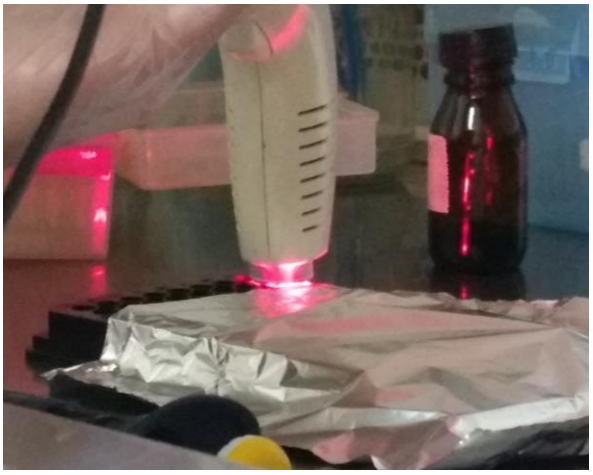

Figure 3. Treatment being performed with the red LED device.

Eight groups were assessed in the study, differing according to the treatment received, as exposed in table 1. A 620-630 nm LED with 300 mW of power was used as the light source.

Methylene blue purchased from Sigma-Aldrich® was diluted in mili-q water and transferred to the plate wells so that the final concentration of the dye was 100 µM.

Table 1. Analysis groups.

Group	Description
G1	Control: mili-q H_2O
G2	Control: saline solution
G3	MO[(1)] +Light
G4	MO[(1)] +dye
G5	MO[(1)] +aPDT (30")
G6	MO[(1)] +aPDT (1')
G7	MO[(1)] +aPDT (2')
G8	MO[(1)] +aPDT(4')

[(1)] Microorganism (*E. coli*)

3. RESULTS AND DISCUSSION

Figure 4 shows the Petri plates after 24h of incubation for the different studied groups. Figure 5 shows the results in CFU/mL.

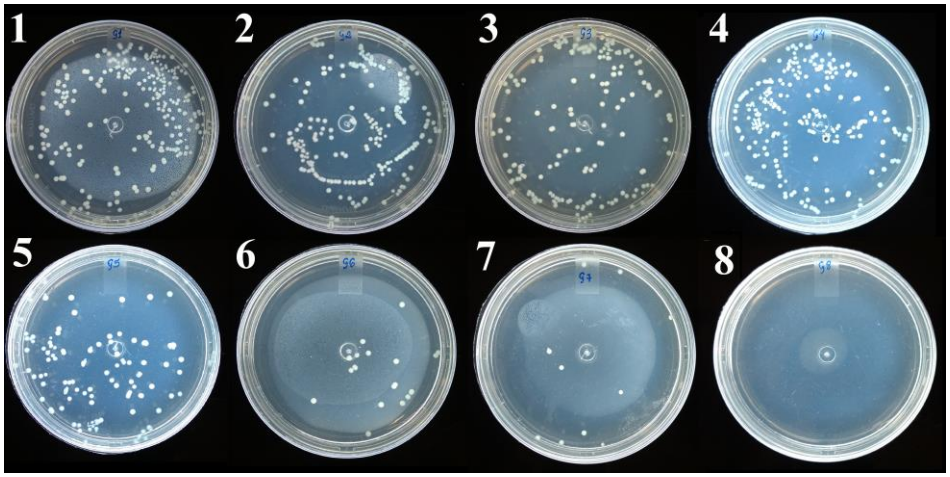

Figure 4. Petri plates from the aPDT procedure with *E. coli*. (1) MO+mili-q water (control); (2) MO+saline (control); (3) MO+light (control); (4) MO+dye (control); (5) MO+aPDT 30''; (6) MO+aPDT 1'; (7) MO+aPDT 2'; (8) MO+aPDT 4'.

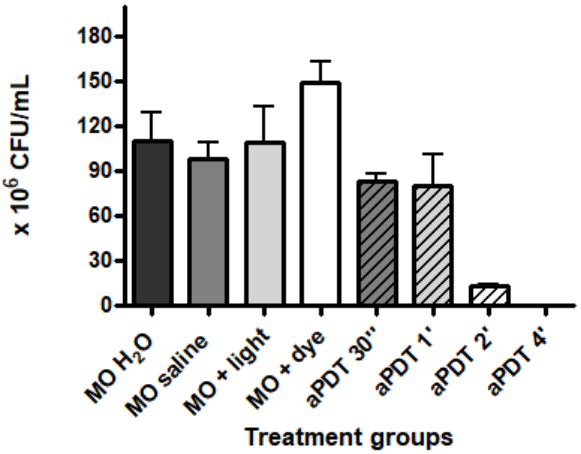

Figure 5. Results for the aPDT procedure in CFU/mL.

The aPDT results showed a clear elimination of *E. coli* in the 4-minute treatment. In a Friedman test, the hypothesis that all means are equal is rejected for this treatment in all comparisons to the control groups. This is the only result that meets the criterion in Brazilian regulation that defines the maximum concentration of *E. coli* in class 1 rivers as 2

CFU/mL (CONAMA 357, 2005). The first four groups did not show any reduction in *E. coli* population, which was expected since no treatment was taking place.

This treatment serves as a guide for the future experiments that will be made with both prototype units, either with bacteria or organic contaminants. It can direct the efforts towards the definition of the optimal conditions that will perform the degradation with the highest efficiency: the highest degradation with the minimum energy and time required. From the units proposed, new sets of experiments with different ECs or even microorganisms and different PSs can be adjusted accordingly, making changes whenever needed, for instance, using different light sources for the respective absorbance of the PSs.

4. CONCLUSION

The preliminary results for *E. coli* show that the aPDT has a solid efficacy, and it is expected that future experiments will help analyse the performance of the prototype units for both microorganisms and ECs. We hope that this and future works will contribute to the studies in pathogens elimination and ECs' treatment. Apart from allowing a closer understanding of the PDI in a flow situation, the prototype units have a potential to shape future WWT plants or to be important assets in existing plants.

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