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Inactivation of Antibiotic Resistant Bacteria in Hospital Wastewater by TiO_2/H_2O_2 Photocatalysis

Raúl B. Aranciaga Pajuelo[a], Jonathan P. Vargas López[a], Carlos A. Castañeda Olivera[a,*], Jorge L. Jave Nakayo[a,b], Elmer G. Benites Alfaro[a], Carlos F. Cabrera Carranza[a,b]

[a] Universidad César Vallejo, Campus Los Olivos, Lima, Peru
[b] Universidad Nacional Mayor de San Marcos, Lima, Perú
caralcaso@gmail.com

Contamination of wastewater by drug-resistant bacteria is an emerging problem because it can have a direct effect on human health and the environment. Therefore, this research determined the photocatalytic efficiency of TiO_2 and H_2O_2 in the inactivation of *Escherichia coli* (*E. coli*) and intestinal *Enterococci* (intestinal *E.*) bacteria. The tests were carried out in two photoreactors of 12 L each, achieving a percentage of efficiency of 75.90% and 93.01% in the inactivation of *Escherichia coli* and *intestinal Enterococci*, respectively, with a dose of 500 mg/L of TiO_2 and 100 mg/L of H_2O_2. In addition, a higher percentage of efficiency in the inactivation of intestinal *Enterococci* was evidenced with respect to *Escherichia coli*, and the chemical and physical parameters of the wastewater improved with photocatalytic treatment, reaching values below the Maximum Allowable Values for non-domestic wastewater discharges. Finally, the results obtained showed that photocatalysis as a wastewater treatment method for the inactivation of antibiotic-resistant bacteria is efficient and could be used as an alternative to treat hospital wastewater.

1. Introduction

The contamination of wastewater by *Escherichia coli* and *intestinal Enterococci* resistant to antibiotics is due to the indiscriminate use of antibiotics which have endowed resistance towards them, causing a negative environmental impact (Proia et al., 2018). According to the WHO (2018), resistance to antibiotics by bacteria is one of the biggest problems for public health. On the other hand, wastewater from hospital and urban areas, as well as wastewater treatment plants are points of generation of resistance to antibiotics (Kordatou, Karaolia and Kassinos, 2018).

Different studies proved that advanced oxidation processes, specifically photocatalytic processes, can inactivate the bacterial reproductive capacity or eliminate them as a consequence of the process (Giannakis et al., 2018; Ttofa et al., 2019). The main function of photocatalysis in the inactivation of *E. coli* and *intestinal Enterococci*, is to eliminate and/or inhibit the reproductive properties of these bacteria, through photocatalytic reactions induced by the absorption of photons of light which can be from a natural or artificial source. In this photocatalytic reaction, the energy of the photons is absorbed by an electron from the valency band, which is promoted to the conduction band of the semiconductor, generating reactive oxygen species such as ^{-}OH radicals, which degrade organic compounds in a non-selective way, thereby microbial growth is inhibited damaging the cell membrane or breaking DNA chains (Moreira et al., 2018; Ameta et al., 2018).

Previous investigations used TiO_2 and H_2O_2 as photocatalysts under strong ultraviolet radiation, demonstrating an inactivation level greater than 80%, obtaining regrowth levels below the samples without treatment, after treatment by photocatalysis (Guo et al., 2017). An important aspect related to the treatment is the residual persistence of the disinfection effect after the treatment because the damaged bacteria can regrow under suitable conditions (Fiorentino et al., 2015). In Peru, hospital wastewater is drained directly without prior treatment, taking into account D.S N°. 021-2009-Vivienda, which indicates the Maximum Admissible Standards for physical and chemical parameters, but not microbiological parameters.

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The use of photocatalysis for the degradation and / or inactivation of bacteria resistant to antibiotics, appears as a promising solution, and with the aim of achieving lower operating costs in the treatment of hospital wastewater, and to prevent the dissemination of these bacteria in the environment (Zhou et al., 2020; Vaiano et al., 2017). That is why the present research is based on the principles of microbial inactivation by photocatalysis using TiO_2 and H_2O_2 with the main objective of determining the efficiency of photocatalytic treatment in the inactivation of *E. coli* and *intestinal E.* resistant to antibiotics in wastewater from the Naval Medical Center.

2. Materials and methods

2.1 Sample collection

The samples came from the Naval Medical Center, Callao - Peru, collecting two samples of 1100 mL of water in the sewer network (272416 E and 8665953 N), to identify and quantify *E. coli* and intestinal *E.*, as well as to analyze the physical parameters and starting chemicals. Likewise, the two photoreactors were filled with 12 L of wastewater each.

2.2 Photoreactor Construction

The photoreactor is made of four transparent acrylic tubes, pipes, and a 12 L container attached to a 0.5 hp water transport pump, which assembled a closed circuit ensuring the homogenization of the photocatalysts with the wastewater to be treated. As shown in Figure 1, we are based on (Blanco, 2002).

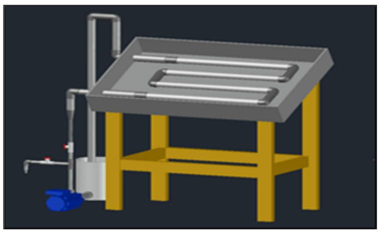

Figure 1: Photoreactor

2.3 Antibiotic Resistance Analysis

The antibiotic resistance test according to Hudzicki, (2009) was applied. For this, Mueller Hinton agar and discs impregnated with antibiotics (penicillin, ampicillin and ciprofloxacin) were used, and the inhibition halos were measured using a vernier. Table 1 shows the level of sensitive, intermediate sensitive or resistant to the antibiotics.

Table 1. Inhibition halos

Antibiotic	Disk content (µg)	Diameter of the inhibition halo in mm *(Escherichia coli)*			Diameter of the inhibition halo in mm (Intestinal *Enterococci)*		
		Resistant	Intermediate	Sensitive	Resistant	Intermediate	Sensitive
Penicillin	10	≤ 28	---	≥ 29	≤ 14	---	≥ 15
Ampicillin	10	≤ 13	14 -16	≥ 17	≤ 16	---	≥ 17
Ciprofloxacin	5	≤ 15	16 - 20	≥ 21	≤ 15	16 -20	≥ 21

2.4 Photocatalysis process

For the photocatalysis process, 250 and 500 mg/L of TiO_2 were used with 50 and 100 mg/L of H_2O_2, with experimentation times of 90 and 180 minutes to determine the best inactivation time of *E. coli* and intestinal *E.* and the effect on physical and chemical parameters.

2.4.1 Quantification of *Escherichia coli* and intestinal *Enterococci*

The methodology proposed by the standard methods for the examination of water and wastewater was followed, (Baird, Eaton and Rice, 2017). For *E. coli*, 1.981 g of McConkey agar was prepared in 40 mL of distilled water and sterilized in an autoclave for one hour. Then, the solution was poured into Petri dishes (20 mL), then the microorganisms were inoculated and incubated at 37 °C for 24 hours. Finally, it was calculated from the colony forming units (CFU/100mL), using the following Equation 1.

$$CFU/100mL = \frac{C.e\ (mean)}{100\ ml} * Dilution\ factor \quad (1)$$

C.e = Colonies enumerated

For intestinal *Enterococci*, 100 mL of Azide Dextrose (AD) broth culture medium was used, put in 10 fermentation tubes and verified pH in the range of 7.2 ± 0.2. 10 mL of the residual water sample was inoculated in each of the tubes with the AD broth and incubated at 35 ° C for 48 hours. Check if there was turbidity, if this is the case, continue with the analysis, but if the sample is not discarded. Transfer the presumptive tubes to Petri dishes with bile esculin agar; previously verify that the pH is between 7.1 ± 0.2; incubate at 35 ° C for 24 hours. The blackish colonies with brown halos are transferred to brain heart infusion (BHI) broth with 6.5% NaCl and incubated at 35°C for 48 hours. Finally, calculate the most probable number (MPN), Equation 2.

$$MPN/100\ mL = \frac{Table\ MPN}{100\ ml} * \frac{10}{v} \quad (2)$$

v = Sample volume of the lowest selected dilution

2.4.2 Physical and Chemical Analysis

For the physical and chemical analysis, the methodology proposed by the standard methods for the examination of water and wastewater (Baird, Eaton and Rice, 2017) was used. The parameters analysed were pH, Oils and Fats (O and F), Biological Oxygen Demand (BOD_5), Chemical Oxygen Demand (COD), Total Suspended Solids (TSS) and Temperature (T°), which were compared with the Maximum Permissible Values (MPV) established by the Ministerio de Vivienda Construcción y Saneamiento (2009) in in Supreme Decree No. 021-2009-VIVIENDA. These average values are: pH (6 - 9), O and F (100 mg/L), BOD_5 (500 mg/L), COD (1000 mg/L), TSS (500 mg/L) and T° (<35 ° C).

2.5 Inactivation efficiency

To determine the percentage of efficiency, the concentrations of the microorganisms before and after the treatment were considered. For this, Equation 3 was used, in which the initial and final data of *E. coli* and *intestinal E.* will be taken, in order to calculate the efficiency (Valencia et al., 2012). All bacterial inactivation tests were performed in triplicate.

$$\frac{Inicial\ C. - Final\ C.}{Inicial\ C.} * 100 \quad (3)$$

Where: Initial C.: Initial concentration and *Final C.*: Final concentration

3. Results and discussion

3.1 Efficiency level in the inactivation of *Escherichia coli* and intestinal *Enterococci*

The level of efficiency in the inactivation of Escherichia coli and intestinal *Enterococci* for each dose is shown in Figure 2.

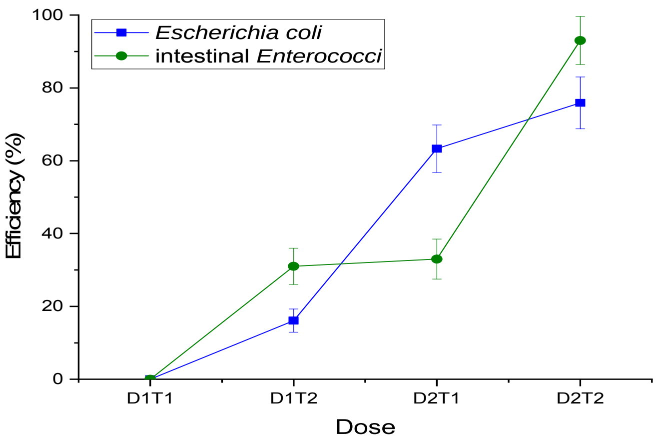

Figure 2: Percentage of inactivation efficiency: D1: 250 and 50 mg/L of TiO_2 and H_2O_2, respectively; D2: 500 and 100 mg/L of TiO_2 and H_2O_2, respectively; T1: 90 minutes; T2: 180 minutes

As shown in Figure 2, for dose D1 at time T1, no efficacy was shown for both bacteria in all three tests, it could be associated with low solar radiation during experimentation, which did not lead the photocatalytic reaction inactivate the *E. coli* and *intestinal E*. (Sillanpää, 2020), as well as the morphological conditions of each drug-resistant bacteria, which can endow them with a certain resistance to treatment (Serna et al., 2019; Sharma et al., 2016), but as the reaction time T2 increased in all three tests, a percentage of 16.1% and 31% is appreciated for *E. coli* and *intestinal E.* respectively. Likewise, for the D2 dose after a T1 reaction time in all three tests, it had an efficiency level of 63.3% and 33% for *E. coli* and *intestinal E*., respectively, but by increasing the T2 reaction time, the highest percentage of efficiency was gotten in both cases reaching 75.9% and 93.01% for *E. coli* and *intestinal E.,* respectively, in all three tests. These results coincide with previous works on the inactivation of drug-resistant bacteria by TiO_2 and H_2O_2, showing that the treatment has a good performance (Maniakova et al., 2020).

For all the doses worked, the inactivation of *E. coli* and intestinal *E*. had an increasing trend, and the highest inactivation efficiency was achieved with the D2 dose at time T2, being higher for intestinal *E.* bacteria. This difference may be due to the fact that *E. coli* has a more resistant structure that does not allow the exchange of solutes with the medium (Giannakis et al., 2018).

3.1.2. Minimum time and effective dose

The minimum time and effective dose were evaluated as a function of the decrease in bacterial concentration of *Escherichia coli* and intestinal *Enterococci*, as shown in Figure 3.

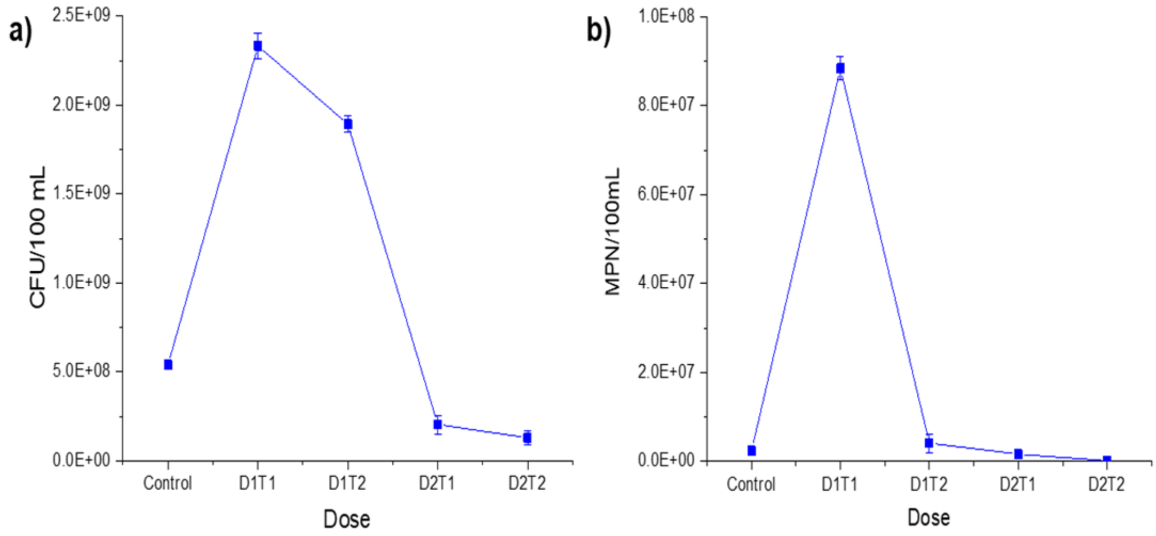

Figure 3. Behaviour of the microorganisms during treatment: a) E. coli (5,40 E+8 CFU/100mL - Control) and b) intestinal Enterococci (2,40 E+06 MPN/100 mL - Control)

From Figure 3 it is observed that the inactivation of the bacteria occurs mainly in time T1 when applying the D2 dose, determining that at a dose of 500 mg/L of TiO_2 and 100 mg/L of H_2O_2 with a reaction time of 180 minutes are the minimum parameters to initiate bacterial inactivation for both *Escherichia coli* and intestinal *Enterococci.* In comparison, Pantoja et al. (2015) achieved total inactivation of *E. coli* after 20 min of photocatalysis treatment (UV-C/TiO_2/SiO_2). This difference in inactivation values is due to the type of wastewater and treatment used (Tiwari, Drogui and Tyagi, 2020). In addition, in Figure 3 it was also observed that the inactivation level initially increased by adding more TiO_2 and H_2O_2, based on the fact that increasing the dose of photocatalysts increased the number of reactive oxygen species such as hydroxyl ($^{-}$OH) radicals, which facilitated bacterial inactivation (Mun et al., 2016). However, it is known that by exceeding doses of photocatalysts greater than 1 g/L, the turbidity of the wastewater to be treated would increase and consequently inhibit the photocatalytic effect of TiO_2 and H_2O_2, this is because the photons of light from ultraviolet rays, would not properly affect the particles of photocatalysts, reducing the efficiency of inactivation (White, 2002).

3.1.3. Sensitivity level

The level of sensitivity of *E. coli* and intestinal *E.* during treatment was determined. According to Figure 2, it was possible to analyse that intestinal *E.* are more sensitive with regard to *E. coli* after photocatalytic treatment, showing the highest percentage of efficiency in their inactivation, reaching values above 90%, being this bacterium considered the most sensitive to treatment. Comparing to the researches of Giannakis et al. (2018), Serna et al. (2019) and Ttofa et al. (2019), they obtained an efficiency percentage greater than 90% for *E. coli*, which worked under pH levels of 5.8 and the samples came from the biological treatment of a wastewater treatment plant (WWTP).

3.1.4. Evaluation of the physical and chemical parameters with the Maximum Permissible Values

For the evaluation of the physical and chemical parameters, the pre- and post-test results of each parameter studied were considered. These values were compared with the maximum permissible values (MPV) established by the Peruvian Ministry of Housing, Construction and Sanitation. See Table 2.

Table 2: Results of the physical and chemical analyses

Parameter	Unit	Pre Test	Post Test	MPV
pH	1 -14	6.26	7.28	6 – 9
BOD_5	mg/L	286	241	500
COD	mg/L	344	316	1000
TSS	mg/L	270	266	500
O and F	mg/L	109	68	100
Temperature	°C	21,1	21	< 35

As shown in Table 2, the photocatalytic treatment had a positive effect on the physical and chemical parameters, since the post-test results did not exceed the Maximum Permissible Values (Ministerio de Vivienda, Construcción y Saneamiento, 2009), but instead decreased compared to the results before treatment, determining that the treatment in question improves the parameters established by the standard. It should be noted that the values of Oils and Fats had a significant decrease, taking into account that the photocatalytic reaction triggers oxide reduction reactions, degrading the organic compounds present in the wastewater (Ameta et al., 2018; Vaiano et al., 2016).

4. Conclusions

The performance of the photocatalytic treatment using TiO_2 and H_2O_2 for the inactivation of *Escherichia coli* and *intestinal Enterococci*, was efficient, obtaining inactivation percentages for *E. coli* over 70% and for *intestinal E.* over 90%, evidencing the minimum treatment time of 90 minutes with an effective dose of 500 mg /L of TiO_2 and 250 mg/L of H_2O_2. On the other hand, of the two study bacteria, the *intestinal E.* was the most susceptible in regard to *E. coli* to photocatalytic treatment. Finally, the applied treatment had positive effects on the physicochemical parameters of the wastewater, complying with the Peruvian standard of Maximum Permissible Values, for non-domestic wastewater discharges.

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