

Inactivation of antibiotic resistant bacteria in hospital wastewater by TiO₂/H₂O₂ 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

*caralcaseo@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 TiO₂ and H₂O₂ 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 TiO₂ and 100 mg/L of H₂O₂. 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 TiO₂ and H₂O₂ 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.

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 TiO₂ and H₂O₂ with the main objective of determining the efficiency of photocatalytic treatment in the inactivation of *E. coli* and intestinal *E. coli* 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. coli*, 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).

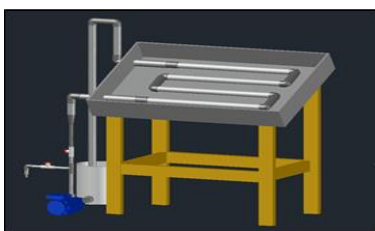


Figure 1: Photoreactor

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 (<i>Escherichia coli</i>) | | | Diameter of the inhibition halo in mm (Intestinal <i>Enterococci</i>) | | |
|---------------|-------------------|---|--------------|-----------|--|--------------|-----------|
| | | 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 TiO₂ were used with 50 and 100 mg/L of H₂O₂, with experimentation times of 90 and 180 minutes to determine the best inactivation time of *E. coli* and intestinal *E. coli* 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 $35^\circ C$ 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 $35^\circ C$ for 24 hours. The blackish colonies with brown halos are transferred to brain heart infusion (BHI) broth with 6.5% NaCl and incubated at $35^\circ C$ 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 $T^\circ (<35^\circ C)$.

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.

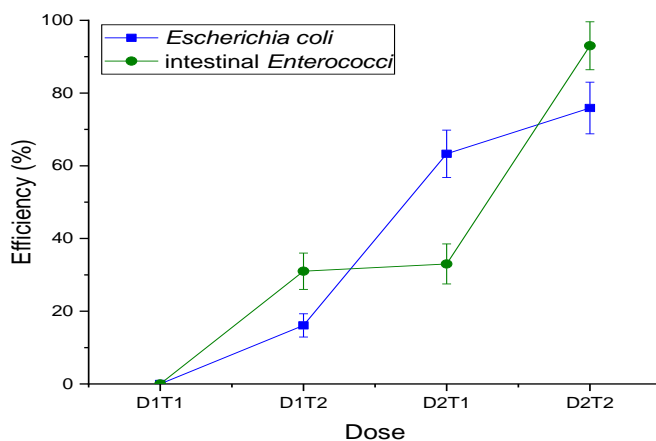


Figure 2: Percentage of inactivation efficiency: D1: 250 and 50 mg/L of TiO_2 and H_2O_2 , respectively; D2: 500 and 100 mg/L of TiO_2 and H_2O_2 , respectively; T1: 90 minutes; T2: 180 minutes

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 TiO₂ and H₂O₂, 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.

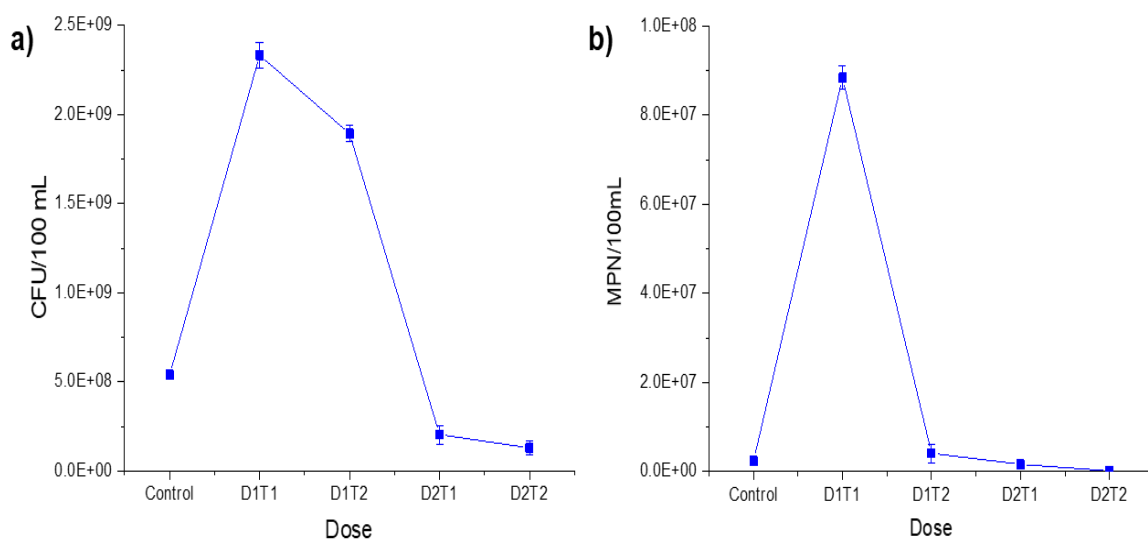


Figure 3. Behaviour of the microorganisms during treatment: a) *E. coli* (5,40 E+8 CFU/100mL - Control) and b) *intestinal Enterococci* (2,40 E+06 MPN/100 mL - Control)

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 TiO₂ and 100 mg/L of H₂O₂ 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/TiO₂/SiO₂). 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 TiO₂ and H₂O₂, 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 TiO₂ and H₂O₂, 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 Tfofa 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 TiO₂ and H₂O₂ 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 TiO₂ and 250 mg/L of H₂O₂. 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.

References

- Ameta, R., Solanki, M.S., Benjamin, S., Ameta, S.C., 2018, Photocatalysis, Advanced Oxidation Processes for Wastewater Treatment: Emerging Green Chemical Technology DOI: 10.1016/B978-0-12-810499-6.00001-2.
- Baird, R., Eaton, A., Rice, E., 2017, Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington DC.
- Blanco, J., 2002, El reactor solar fotocatalítico: Estado del arte, Solar Safe Water, Almería - España, pp. 277–302.
- Florentino, A., Ferro, G., Alferéz, M.C., Polo-López, M.I., Fernández-Ibañez, P., Rizzo, L., 2015, Inactivation and regrowth of multidrug resistant bacteria in urban wastewater after disinfection by solar-driven and chlorination processes. J. Photochem. Photobiol. B Biol. 148, 43–50 DOI: 10.1016/j.jphotobiol.2015.03.029.
- Giannakis, S., Voumard, M., Rtimi, S., Pulgarin, C., 2018, Bacterial disinfection by the Photo-Fenton process: Extracellular oxidation or intracellular photo-catalysis? Appl. Catal. B Environ, 227, 285–295 DOI: 10.1016/j.apcatb.2018.01.044.
- Guo, C., Wang, K., Hou, S., Wan, L., Lv, J., Zhang, Y., Qu, X., Chen, S., Xu, J., 2017. H₂O₂ and/or TiO₂ photocatalysis under UV irradiation for the removal of antibiotic resistant bacteria and their antibiotic resistance genes. J. Hazard. Mater. 323, 710–718 DOI: 10.1016/j.jhazmat.2016.10.041.
- Hudzicki, J., 2009, Kirby-Bauer Disk Diffusion Susceptibility Test Protocol 1–23, <www.asm.org/getattachment/2594ce26-bd44-47f6-8287-0657aa9185ad/Kirby-Bauer-Disk-Diffusion-Susceptibility-Test-Protocol-pdf.pdf> accessed 15.05.2020.

- Kordatou, M., Karaolia, P., Kassinos, D., 2018, The role of operating parameters and oxidative damage mechanisms of advanced chemical oxidation processes in the combat against antibiotic-resistant bacteria and resistance genes present in urban wastewater, *Water Res.*, 129, 208–230 DOI: 10.1016/j.watres.2017.10.007.
- Maniakova, G., Kowalska, K., Murgolo, S., Mascolo, G., Libralato, G., Lofrano, G., Sacco, O., Guida, M., Rizzo, L., 2020, Comparison between heterogeneous and homogeneous solar driven advanced oxidation processes for urban wastewater treatment: Pharmaceuticals removal and toxicity. *Sep. Purif. Technol.* 236, 116249 DOI: 10.1016/j.seppur.2019.116249.
- Ministerio de Vivienda Construcción y Saneamiento, 2009, Valores Máximos Admisibles de las aguas residuales no domésticas en el sistema de alcantarillado sanitario, Decreto Supremo N° 021-2009-VIVIENDA, Diario El Peruano, Perú, <www.sosanit.pe/pdf/documentoprovma.pdf> accessed 25.03.2020.
- Moreira, N.F.F., Narciso-da-Rocha, C., Polo-López, M.I., Pastrana-Martínez, L.M., Faria, J.L., Manaia, C.M., Fernández-Ibáñez, P., Nunes, O.C., Silva, A.M.T., 2018. Solar treatment (H₂O₂, TiO₂-P25 and GO-TiO₂ photocatalysis, Photo-Fenton) of organic micropollutants, human pathogen indicators, antibiotic resistant bacteria and related genes in urban wastewater, *Water Res.*, 135, 195–206 DOI: 10.1016/j.watres.2018.01.064.
- Mun, K., Wei, C., Sing, K., Ching, J., 2016, Recent developments of zinc oxide based photocatalyst in water treatment technology: A review, *Water Res.*, 88, 428–448
- World Organization Health Datos recientes revelan los altos niveles de resistencia a los antibióticos en todo el mundo, 2018 <www.who.int/mediacentre/news/releases/2018/antibiotic-resistance-found/es/> accessed 30.07.2020.
- Pantoja, J.C., Proal, J.B., García, M., Cháirez, I., Osorio, G.I., 2015, Eficiencias comparativas de inactivación de bacterias coliformes en efluentes municipales por fotólisis (UV) y por fotocatalisis (UV/TiO₂/SiO₂), Caso: Depuradora de aguas de Salamanca, España, *Rev. Mex. Ing. Quim.*, 14, 119–135.
- Proia, L., Adriana, A., Jessica, S., Carles, B., Marinella, F., Marta, L., Luis, B.J., Servais, P., 2018, Antibiotic resistance in urban and hospital wastewaters and their impact on a receiving freshwater ecosystem. *Chemosphere* 206, 70–82 DOI: 10.1016/j.chemosphere.2018.04.163.
- Serna-Galvis, E.A., Troyon, J.A., Giannakis, S., Torres-Palma, R.A., Carena, L., Vione, D., Pulgarin, C., 2019, Kinetic modeling of lag times during photo-induced inactivation of *E. coli* in sunlit surface waters: Unraveling the pathways of exogenous action. *Water Res.* 163, 114894 DOI: 10.1016/j.watres.2019.114894.
- Sharma, V.K., Johnson, N., Cizmas, L., McDonald, T.J., Kim, H., 2016, A review of the influence of treatment strategies on antibiotic resistant bacteria and antibiotic resistance genes. *Chemosphere* 150, 702–714 DOI: 10.1016/j.chemosphere.2015.12.084.
- Tiwari, B., Drogui, P., Tyagi, R.D., 2020, Multidrug-resistant genes and pathogenic bacteria in hospital wastewater, *Current Developments in Biotechnology and Bioengineering*. BV, <[dx.doi.org/10.1016/B978-0-12-819722-6.00006-7](https://doi.org/10.1016/B978-0-12-819722-6.00006-7)> accessed 20.07.2020.
- Ttofa, L., Raj, S., Prakash, H., Fatta-Kassinos, D., 2019, Solar Photo-Fenton oxidation for the removal of ampicillin, total cultivable and resistant *E. coli* and ecotoxicity from secondary-treated wastewater effluents. *Chem. Eng. J.* 355, 91–102 DOI: 10.1016/j.cej.2018.08.057.
- Valencia, E., Aragón, R.A., Romero, J., Asociado, M.S.P., Surcolombiana, U., Agrícola, D.D.I., 2012, Planta de tratamiento de aguas residuales de nátaga en cultivo de cacao (*Theobroma cacao* L.) potential reuse of effluent from the nátaga municipality wastewater treatment plant for the crop of cocoa (*Theobroma cacao* L.) 15, 77–86 < www.scielo.org.co/scielo.php?script=sci_abstract&pid=S0123-42262012000100009> accessed 24.02.2020.
- Vaiano V., Matarangolo M., Sacco O., Sannino D., 2017, Photocatalytic Removal of Eriochrome Black T Dye over ZnO Nanoparticles Doped with Pr, Ce or Eu, *Chemical Engineering Transactions*, 57, 625-630.
- Vaiano V., Sacco O., Sannino D., Stoller M., Ciambelli P., Chianese A., 2016, Photocatalytic removal of phenol by ferromagnetic n-tio2/sio2/fe3o4 nanoparticles in presence of visible light irradiation, *Chemical Engineering Transactions*, 47, 235-240 DOI: 10.3303/CET1647040
- Zhou, C. shuang, Wu, J. wen, Dong, L. li, Liu, B. feng, Xing, D. feng, Yang, S. shan, Wu, X. kun, Wang, Q., Fan, J. ning, Feng, L. ping, Cao, G. li, 2020, Removal of antibiotic resistant bacteria and antibiotic resistance genes in wastewater effluent by UV-activated persulfate. *J. Hazard. Mater.*, 388, 122070 DOI: 10.1016/j.jhazmat.2020.122070.