

EMPLOYMENT OF PHOTOLYTICAL AND PHOTOCATALYTICAL METHODOLOGIES FOR REMOVAL OF CEPHALEXIN IN AQUEOUS SOLUTION



EMPREGO DE METODOLOGIAS FOTOLÍTICAS E FOTOCATALÍTICAS PARA A REMOÇÃO DE CEFALEXINA EM SOLUÇÃO AQUOSA

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RESUMO

A crescente contaminação dos corpos d'água pela presença de ativos farmacêuticos presentes em medicamentos de uso humano e veterináriotem levado a sérias consequências ambientais, tal comoo surgimento de cepas bacterianas resistentes.Os antibióticos β -lactâmicos, classe da qual faz parte a cefalexina (CPX), é um dos contaminantes mais frequentemente encontrados em águas naturais. Objetivando determinar as melhores condições para a fotodegradação deste ativo em solução aquosa foram investigados os métodos fotolíticoe fotocatalítico. Para os experimentos fotocatalíticos empregando catalisadores homogêneo (H₂O₂) e heterogêneo (TiO₂), os parâmetros foram organizados de acordo com um planejamento experimental 2³, com objetivo de observar a influência de cada fator no processo, assim como a interação entre eles nos diferentes pHs estudados. Da análise dos resultados foi possível verificar que os parâmetros pH, H₂O₂, assim como a interação entre os parâmetros H₂O₂ e TiO₂ são significantes para o processo, e que as melhores condições para a fotocatáliseforam pH 4, [TiO₂] = 50 mg L⁻¹ e [H₂O₂] = 1000 mg L⁻¹. No ensaio de atividade antimicrobiana foi observado que somente duas cepas, *E. coli* e *A. hydrophila*, não apresentaram sensibilidade ao fármaco fotoquimicamente tratado, mostrando que nesses casos o ativo farmacêutico teve sua atividade antimicrobiana diminuída.

Palavras-chave: cefalexina, fármacos no ambiente, fotodegradação.

ABSTRACT

The increasing contamination of water bodies by the presence of pharmaceutical actives present in medicinal products for human and veterinary use has led to serious environmental consequences, such as the emergence of resistant bacterial strains. β -lactam antibiotics, a class of which is cephalexin (CPX), is one of the most commonly found contaminants in natural waters. Aiming to determine the best conditions for the photodegradation of this active in aqueous solution, the photolytic and photocatalytic methods were investigated. For the photocatalytical experiments employing homogeneous (H₂O₂) and heterogeneous (TiO₂) catalysts, the parameters were organized according to an experimental design 2³, with the objective of observing the influence of each factor in the process, as well as the interaction between them in the different pHs studied. From the analysis of the results it was possible to verify that the parameters pH, H₂O₂, as well as the interaction between the parameters H₂O₂ and TiO₂ are significant for the process, and that the best conditions for the photocatalysiswere pH 4, [TiO₂] = 50 mg L⁻¹ and [H₂O₂] = 1000 mg L⁻¹. In the antimicrobial activity assay, only two strains, *E. coli* and *A. hydrophila*, showed no sensitivity to the photochemically treated drug, showing that in these cases the pharmaceutical active had its antimicrobial activity decreased.

Keywords: Cephalexin, pharmaceuticals in the environment, photodegradation.

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INTRODUCTION

The contamination of aquatic environments undesirable effects causes to the The contamination of aquatic environments causes undesirable effects to the ecosystem biota, endangering the quality of the water and the abundance of some organisms. The pollution source can have its origin in many anthropogenic activities, which may be evidenced by the presence of emerging contaminants in aquatic environments. Emerging contaminants are those that point out in the recent detection of products, for example, pharmaceuticals, personal care products, plasticizers and pesticides(HALLING-SORENSEN et al., 1998; FENT. WESTON, CAMINADA, 2006; KÜMMERER, 2009a).

Pharmaceutical products are largely used in human and veterinary medicine. The presence of these products in surface water and groundwater consists of a major concern factor in environmental pollution since it can be harmful to the quality of the water resources due to the persistence, bioaccumulation and toxicity of these compounds (AY & KARGI, 2010; KHETAN & COLLINS, 2007).

The residues of pharmaceuticals may have different sources, that is, from effluents of pharmaceutical industries, rural effluents, inadequate disposal of expired medicines, due to veterinary use, and mainly to the domestic sewage released in water resources (BILA & DEZOTTI, 2003; KÜMMERER, 2009b).

Pharmaceutical residues have may antimicrobial properties complex or even structures that are not susceptible to The conventional process for biodegradation. treating industrial and domestic effluents, based on biological degradation, allows partial removal these contaminants. resulting of in the contamination of surface water, groundwater and sediments (NOGUEIRA; MELO; TROVO, 2008; PALOMINOS, 2009).

The CPX, a β -lactam antibiotic (Figure 1), which belongs to the first-generation of cephalosporin drugs, is widely used in human and veterinary medicine (REYNOSO *et al.*, 2012). These drugs act interrupting the synthesis of the bacterial cell wall, which stops its growth (WANG & LIN, 2012). Studies have shown that, among the contaminants of pharmacological origin, approximately 15% of the pharmaceuticals found in the environment belong to the class of antibiotics (ARAUJO *et al.*, 2012).



FIGURE 1. Structural formula of the antibiotic cephalexin (CPX).

The concentrations found in water bodies are considerably smaller than those intentionally consumed, and vary in the limits of μ gL⁻¹ and ngL⁻¹ (BILA & DEZOTTI, 2003). Despite this, a long-term exposure as well as the combination of these pharmaceuticals with other pollutants may become a potential risk to the aquatic and terrestrial organisms (ABELLÁN; GIMÉNEZ; ESPLUGAS, 2009).

The presence of antibiotics in the aquatic environment for a long period, even at low concentrations, result in different effects that can be harmful to the environmental balance. For example, these pharmaceuticals in the environment are not enough to inhibit or kill all the bacteria sensitive to the antibiotic, herewith, according to some studies, being able to contribute to the development of bacterial resistance to antibiotics(KÜMMERER, 2009b).

In this sense. photolytical and photocatalytical processes are studied in order to verifv its efficiency for insertion to the methods. The photolytical conventional processes employ light as a single degradation source and usually have low efficiency, while the photocatalytical processes are based on the use of homogeneous or heterogeneous catalysts, or an association of both, aiming to increase the reaction rates (TEIXEIRA & JARDIM, 2004).

Techniques employing oxidants such as hydrogen peroxide (H_2O_2) and ozone (O_3) are characterized by the formation of hydroxyl radicals (HO), and thus, are called Advanced Oxidation Processes (AOPs), which have an increased efficiency when subjected to UV irradiation and in the presence of catalysts (metallic ions or semiconductors) (MELO *et al.*, 2009; UTRILLA *et al.*, 2013).

Studies indicate that the heterogeneous

photocatalysis in TiO₂ suspension is an important technique of advanced oxidation concerning the removal of contaminants at low concentrations, using artificial or natural light, due to its low cost, commercial availability, non-toxicity and photochemical stability.

Thus, aiming to minimize the environmental presented, the removal of these effects contaminants must be carried out by means of adequate processes of treatment, and, in this sense, we propose the employment of photolytical and photocatalytical methodologies, order to accelerate the reactions of in degradation employing catalysts UV and irradiation.

RESULTS AND DISCUSSION

CHEMICALS AND ANTIBIOTIC

The titanium dioxide was purchased from Sigma-Aldrich. The cephalexin was purchased as an active principle from the local commerce. The sodium hydroxide (NaOH) (FMAIA), hydrochloric acid (HCI) and hydrogen peroxide (Anidrol), all analytical grade, were used without an additional purification.

ANALYTICAL METHODS

The experiments were followed by UV-VIS spectrophotometry, obtaining the absorption spectra at 15 minutes intervals, using an UV-1601PC (Shimadzu) spectrophotometer, scaning from 220 to 390 nm.

The hydrogen peroxide concentration was measured using the permanganometric titration technique, as described in the literature (VOGEL, 1992).

PHOTOCHEMICAL REACTOR

The irradiation experiments were done in lab-made photo-reactor equipped with a highpressure mercury (Hg) vapor lamp with 125 W of power, which emits light over the entire UV-Visible spectrum, and whose bulb was removed to avoid the filter effect of the ultraviolet (UV) radiation. The Hg vapor lamp was installed 11 cm of distance from the sample. Figure 2 presents a scheme of the photo-reactor used for the irradiations, as previously described in Schneider *et al.* (2014).



FIGURE 2. Scheme of the photo-reactor used for the irradiations. A) 125W mercury vapor lamp; B)Beaker with the aqueous solution of the pharmaceutical; C) Inner wall of the reactor; D) Magnetic agitator; E) Wooden support for magnetic stirrer.

SAMPLE PREPARATION

The CPX stock solution was prepared by dissolving 0.2 g of the active principle in 100 mL of distilled water, obtaining a solution with concentration equivalent to 2.0 g L⁻¹ (5.76 x 10⁻³ mol L⁻¹). The solution was subjected to ultrasound bath for 40 minutes to ensure the complete solubilization of the active and the elimination of air bubbles. The best sample concentration to be submitted to irradiation was determined by the construction of the calibration curve. For that, 10 different solutions, ranging from 5 mg L^{-1} to 50 mg L⁻¹ were prepared by dilutions of the CPX stock solution. The concentration whose absorption most approached to unity was choosen as the most appropriate.

For each of the experiments of this work, a volume of 200 mL of the solution was used and irradiated for 120 minutes in the photo-reactor. Aliquots of approximately 3.0 mL were collected every 15 minutes for performing spectral scanings, used to monitor the reaction kinetics.

For the construction of the $ln(A_0/A)$ vs. time plots, were employed the values of the absorption maximum located at the wavelength of 262 nm. The graphs were plotted employing the spreadsheet Microsoft Excel®, and the statistical analysis was performed using the statistical software Statistica®. EXPERIMENTS OF DEGRADATION OF THE PHARMACEUTICAL IN AQUEOUS SOLUTION EMPLOYING UV LIGHT (PHOTOLYTIC METHOD)

Effect of pH

For adjusting the pH at the values of 4, 7 and 10, 0.01 mol L⁻¹ HCl and 0.01 mol L⁻¹ NaOH solutions were used. The solutions initial pH was determined employing a universal combined glass electrode (BNC Eltex) and a pH-meter (Quimis/Q.400.A). The 50 mg L⁻¹ CPX solutions, at the different adjusted pHs, were irradiated for 120 minutes.

EXPERIMENTS OF DEGRADATION OF THE PHARMACEUTICAL EMPLOYING H₂O₂AND UV LIGHT AS CATALYSTS (HOMOGENEOUS PHOTOCATALYTICAL METHOD)

The. hydrogen peroxide concentrations of 500 mgL⁻¹, 1000 mgL⁻¹ and 1500 mgL⁻¹ were employed in the irradiation experiments. Each of the H_2O_2 additions was studied at pHs 4, 7 and 10, in a total of nine distinct experiments.

EXPERIMENTS OF DEGRADATION OF THE PHARMACEUTICAL EMPLOYING H_2O_2 , TiO₂ AND UV LIGHT AS CATALYSTS (HETEROGENEOUS PHOTOCATALYTICAL METHOD)

Aqueous solutions of CPX were prepared containing the required amount of TiO_2 to obtain the previously defined concentrations. The pH was adjusted at the desired values and the mixture was kept in the dark under constant stirring for 30 minutes, until the adsorption equilibrium was reached. Then, the required volume of H_2O_2 was added and the sample was subjected to UV irradiation, collecting aliquots of the irradiated solution at 15 minute intervals. To perform the spectral scanings, the sample was previously filtered in 0.2 µm pore size syringe filters (Chromafil PVDF-20/15 MS), and forthwith transferred to a quartz cuvette of 1.0 cm optical pathlength.

Experimental design 2³

The methodology used in this study for the development of the experimental design 2^3 is described in the literature (BARROS NETO; SCARMINIO; BRUNS, 2007) .The levels of the variables of the process ([TiO₂], [H₂O₂] and pH) were determined from preliminary experimental

tests, which allowed the catalysts concentrations and the initial pH as well as the interaction between them to be better evaluated in the experimental design 2^3 .

In the statistical test, the importance of the photodegradation process operational parameters was assessed. The studied parameters ($[TiO_2]$, $[H_2O_2]$, and initial pH) are displayed in Table 1.

The 11 experiments of the design were performed in a random way. The three variables of the process varied among each other at both two levels (-1 and +1) of concentration of the reagents and initial pH, being performed a triplicate at the center point, in order to increase the degree of freedom of the model, as displayed in Table 2.

The experimental results obtained in the experimental design were assessed by means of the statistical software Statistica® (Statsoft, Inc), in the "experimental design" mode, for two variation levels, through which it was performed analyses of effects, interactions, response surface and analysis of variance (ANOVA).

EVALUATION OF THE PHOTOLYTICAL PROCESS AND THE INFLUENCE OF pH

Photolysis – pH Effect

By analysis of the calibration curve that showed a good linear fit in the concentration range studied ($R^2 = 0.9961$), the concentration of 50 mgL⁻¹ was chosen as the most convenient, because it has abosrvance close to the unit.

Initially was investigated the influence of pH in the CPXphotodegradation process. For that, CPX aqueous solutions were prepared, at the concentration of 50 mgL⁻¹inthe pHs 4, 7 and 10. It was observed that the CFX aqueous solution has its maximum absorbance at the wavelength 262nm.

After 120 minutes of irradiation, the percentage of the absorption reduction (*%AbsRed*) of this band centered at 262 nm was calculated according to Equation 1.

$$\%AbsRed = \left(1 - \left(\frac{A}{A_0}\right)\right) \times 100$$
 (Eq. 1)

where A is the absorbance after 120 min of exposure to UV light and A_0 is the absorbance at the initial time.

In Figure 3, the spectra obtained for the three experiments at zero time can be observed, and it is verified that the absorbance did not vary at any pH. The absorption spectra were monitored at 15-minute intervals, and all of them had a very similar behavior, differing only by the values of *%AbsRed*. At the end of 120 minutes, the *%AbsRed* values of 10.63%, 10.18% and 9.28% were obtained at pH 4, 7 and 10, respectively.

Figure 4 shows the plots of $ln(A_0/A)$ as a function of time. It is possible to verify that pH 4 had the greatest value of k, with $k = 9 \times 10^{-4}$ min⁻¹, which means that CPX is slightely faster rapidly at pH 4 than at pH 7 and 10. The values of correlation coefficient (R²) were calculated for the experiments of pH 4, 7, 10, with adjustments of 0.9809, 0.9912 and 0.9957, respectively. Thus, it is possible to affirm, from the linear fits of $ln(A_0/A)$ *versus* time, that the processes follow a first-order kinetic (or pseudo first-order) up to 120 minutes of reaction.

EVALUATION OF THE PHOTODEGRADATION PROCESS EMPLOYING H₂O₂ AND UV LIGHT

Effect of H₂O₂ concentration

Table 3 presents the *%AbsRed* values for each experiment performed with the addition of H_2O_2 , besides those resulting from the photolysis process, which had no addition of H_2O_2 . For the determination of the rate constant (*k*) according to a first-order (or pseudo first-order) kinetics, graphs of $ln(A_0/A)$ as a function of time were plotted for all experiments, monitoring the behavior of the band centered at 262 nm.

As it can be seen in Table 3, the experiments showed a linear fit of the experimental points. Comparing the experiments, it is observed that the kinetics of the sample degradation with the presence of 1500 mgL⁻¹ of H_2O_2 at pH 4, which was the best performance, has k in the order of $34x10^{-4}$ min⁻¹ (R² = 0.9941), a value 3.4 times superior to that obtained in the photolytical process at same pH 4.

It is also possible to verify, in the same Table 3 that there is a significant decrease in band intensity when compared the values of photolysis (0 mgL⁻¹ of H_2O_2) and photocatalysis employing H_2O_2 . This happens due to the

production of highly reactive hydroxyl radicals (KIM; YAMASHITA; TANAKA, 2009). When performed the addition of 1500 mgL⁻¹ of H_2O_2 , there is a slight loss of efficiency in the process, except for pH 4, which can be explained by the fact that at high concentrations of hydrogen peroxide, reactions consuming hydroxyl radicals may occur, negatively affecting degradation (MELO *et al.*, 2009).

In addition to that, in Table 3 it is also noted that for each pH value employed, a different concentration of H₂O₂ showed a higher degradation percentage, that is, at pH 4 the H_2O_2 concentration which presented the best result was 1500 mgL⁻¹; however at pH 7, the best concentration was 500 mgL⁻¹, while at pH 10, the concentration of 1000 mgL⁻¹ showed a better result. Thus, it can be affirmed that the parameter H_2O_2 is not significant for the process, due to the small variation in %AbsRed when the concentration of this catalyst is changed, a fact that can also be statistically proved.

Figure 5 shows the effects of both variables employed (pH and H_2O_2). It is observed that at acidic pH (4.0) the photodegradation process is more efficient, consequently, only the variable pH is significant for the process.

Figure 6 shows the results of the photocatalytical experiments employing H_2O_2 at the concentration of 1500 mgL⁻¹ and pH 4. It is possible to observe the existence of an isosbestic point centered at approximately 280 nm, indicating that there is the conversion of a structure into another one without the presence of a third form.



FIGURE 5: Response surface for the variables H_2O_2 and pH.

By means of the spectrophotometry technique, it is not possible to determine which

structure is being formed. For that, it would be necessary the use of more sophisticated techniques, such as high performance liquid chromatography coupled with a mass detector (HPLC/MS).

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS OF THE PHOTOCATALYSIS PROCESS EMPLOYING TiO₂, H₂O₂ AND pH

With the addition of the catalyst TiO_2 , there was an increase in the *%AbsRed*. Despite this, higher concentrations of that catalyst do not result in an improvement in the antibiotic degradation, once it decreases the penetration of light, increasing its dispersion, and causes TiO_2 sedimentation (ELMOLLA; CHAUDHURI, 2010).

Тο evaluate the efficiency of the photodegradation process employing artificial irradiation the efficiency %AbsRed was assessed. The obtained results showed variations from 22.6% to 42.0% in the absorbance reduction, as presented in Table 4.

The fit of a linear model to the table data, considering only the significant parameters of the process, results in Equation 2, in which values are presented in a coded manner.

$$\% AbsRed = 33,049 - 3,792. \text{ pH} + 3,658. [H_2O_2] - 2,98. [H_2O_2]. [TiO_2]$$
 (2)

The generated statistical model showed a good fit of the values predicted by the model for absorbance reduction as a function of the observed values, as presented in Figure 7.

From the observation of the data shown in Table 4, we conclude that at a significance level of 95% (p-value < 0,05), the parameters pH, H_2O_2 , as well as the interaction between the parameters H_2O_2 and TiO_2 are significant for the process. Through the values of texp, with degree of freedom equal to 4, the variables pH e H_2O_2 showed a very close degree of influence, but in reverse. For the variable pH, the decrease from level 1 to -1 will cause an increase of the percentage absorbance reduction. While for the variable H_2O_2 , the decrease of its value will cause the decrease of response. From the evaluation of the interaction between the factors H_2O_2 and TiO_2 , it is possible to conclude that the increase of interaction causes the decrease of the response value in a less significant way than the independent factors do.

The experimental responses based on the

values of *%AbsRed*in the experimental design allowed the development of a statistical model with linear fit of the effects of interaction among the coefficients associated with the variables used in the photodegradation process, as shown in Table 5.

The significance of the effects of the operational significant variables and their possible combined actions are verified by applying the analysis of variance (ANOVA), by means of the software Statistica®, as presented in Table 6. In the proposed conditions, the F_{Cal} (24.14) is higher than the F_{Tab} (3; 7; 0.05 = 4.35), therefore, the model is valid for the confidence interval of 95%.

The experimental results obtained from the design and the analysis of the operational parameters of the photodegradation reactor can be better visualized in the response surface N-Dimensional graph. In Figure 8, the effects of both significant variables of the process (pH and H₂O₂) were analyzed, maintaining the third variable (TiO₂) constant at the center point established in the design. It is observed that at acidic pH (4.0), there is a better efficiency of the photodegradation process. The result tends to be even better when the variable H_2O_2 is approached to level 1, where the compound concentration used was equal to de 1000 mgL⁻¹.



FIGURE 8: Response surface remaining fixed at center point (75 mgL^{-1}) to the variable TiO₂.

ANTIMICROBIAL ACTIVITY: DISC-DIFFUSION TEST IN AGAR.

In order to verify the alteration of the antibacterial activity of the cephalexin exposed to the photochemical treatment, the disc-diffusion test against the bacteria: Escherichia coli ATCC 25922, Escherichia coli (IC1), Escherichia coli (IC2), Klebsiella pneumoniae ATCC 13883, Staphylococcus Aureus ATCC 25923, two environmental isolates of Aeromonashydrophila, and Staphylococcus xylosus (environmental isolate). For both sterile filter paper disks of 4 mm in diameter were impregnated with 5 μ L of the compounds under investigation at 6 μ g/ μ L, making a total of 30 μ g of compound per disc.

For inoculauniformization, bacterial cultures were grown for 18-24 h in TSB broth and densities were adjusted by the equivalence to 0.5 tube of the McFarland Scale $(1 \times 10^8 \text{ CFU mL}^{-1})$. After inoculation of the cultures in Mueller-Hinton medium with swab aid, the impregnated discs were placed on the culture medium. After incubation at 35 ° C for 24 hours, the inhibition halos were read in millimeters. The experiment was done in duplicate.

The sensitivity results of the bacteria exposed to untreated CPX and after being phototreated with UV are shown in Table X. It is observed that for all species that showed sensitivity to the antibacterial, there was a change of status to resistant or intermediate or at least decrease Inhibition halo, in some cases with pronounced decrease. These findings indicate loss of compound activity after treatment.

CONCLUSIONS:

We conclude with this study that CPX in aqueous solution, at pH 4, and in the presence of 1000 mgL⁻¹ of H₂O₂, underwent a first order (or pseudo first order) photodegradation process, with rate constant (k) of 28×10^{-4} min⁻¹. This value was 3.5 times superior to that determined for the photolytic process at same pH (k= 8×10^{-4} min⁻¹).

In the experiments employing H_2O_2 and TiO_2 and different pH values, it was possible to verify, through the experimental design 2³, that the parameters pH and H_2O_2 , as well as the interaction between the parameters H_2O_2 and TiO_2 are significant for the process, since the decrease of the variable pH causes a percentage increase of absorbance reduction, while for the variable H_2O_2 , the decrease of the value causes a decrease in response. Concerning the interaction between the factors H_2O_2 and TiO_2 , it follows that the increase in interaction causes the decrease of the value, in a less significant way than the independent factors do.

Antimicrobial tests showed a decrease of CPX activity for two bacterial strains, *E. coli* and *A. hydrophila*. From these, the latter was a strain

isolated from an environmental sample of river Toledo water.

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FIGURE 3: CPX absorbance spectra at initial time at pH 4, 7 and 10.



FIGURE 4: Linear fits of the In(A₀/A) vs. time (min) points for the samples with different pH values.



FIGURE 6:CPX absorbance spectra as a function of 50 mgL⁻¹CPX, 1500 mgL⁻¹H₂O₂ and irradiation time at pH 4.



FIGURE7: Predicted values for the photodegradation process, employing artificial irradiation, as a function of the observed values for percentage of absorbance reduction (%*AbsRed*).

Variable		Levels	
variable	-1	0	1
$TiO_2 (mg L^{-1})$	50	75	100
$H_2O_2(mg L^{-1})$	500	750	1000
pH	4	7	10

TABLE 1: Levels of the variables (TiO₂, H₂O₂ and pH) used in the experimental design.

TABLE 2: Variations of the three concentration levels of reagents and pH.

Experiment —	Experimental conditions				
	рН	H_2O_2	TiO ₂		
P1	1	1	1		
P2	-1	1	1		
P3	1	-1	1		
P4	-1	-1	1		
P5	1	1	-1		
P6	-1	1	-1		
P7	1	-1	-1		
P8	-1	-1	-1		
P9	0	0	0		
P10	0	0	0		
P11	0	0	0		

TABLE 3: Band reduction percentage values, kinetic constants and R^2 values for the homogeneous photocatalysis experiments employing H_2O_2 at different pH concentrations.

р <mark>н</mark> [H ₂ O ₂]		%AbsRed at	k (min ⁻¹)	R^2
	(mgL⁻¹)	262 nm		
	0	10,63	10x10 ⁻⁴	0,9809
٨	500	31,58	31x10⁻⁴	0,9922
4	1000	29,33	28x10 ⁻⁴	0,9922
	1500	33,29	34x10 ⁻⁴	0,9941
	0	10,18	9x10⁻⁴	0,9912
7	500	27,08	26x10⁻⁴	0,9915
/	1000	26,05	26x10⁻⁴	0,9965
	1500	24,57	24x10 ⁻⁴	0,9799
	0	9,28	8x10⁻⁴	0,9957
10	500	15,50	16x10⁻⁴	0,9967
	1000	27,11	26x10⁻⁴	0,9995
	1500	21,51	21x10⁻⁴	0,9756

Experiment	рН	$H_2O_2(mgL^{-1})$	TiO ₂ (mgL ⁻¹)	%AbsRed
P1	10 (1)	1000 (1)	100 (1)	28,24
P2	4 (-1)	1000 (1)	100 (1)	41,02
P3	10(1)	500 (-1)	100 (1)	30,57
P4	4 (-1)	500 (-1)	100 (1)	35,98
P5	10 (1)	1000 (1)	50 (-1)	33,49
P6	4 (-1)	1000 (1)	50 (-1)	41,96
P7	10(1)	500 (-1)	50 (-1)	22,62
P8	4 (-1)	500 (-1)	50 (-1)	34,32
P9	7 (0)	750 (0)	75 (0)	36,07
P10	7 (0)	750 (0)	75 (0)	33,96
P11	7 (0)	750 (0)	75 (0)	32,52

TABLE 4: Results of the analyses of absorbance presented as %AbsRed

TABLE 5: Effect of the interaction between the variables of the photodegradation process employing artificial irradiation for %AbsRed.

Variables and interactions	Effect of Variable	Model coefficient	Coefficient standard error	t _{exp} (4)	<i>p</i> -value
Intercept	33,049	33,049	0,694	47,638	0,000
рН	-7,583	-3,792	0,804	-4,715	0,009
H_2O_2	7,315	3,658	0,804	4,548	0,010
TiO ₂	2,656	1,328	0,867	1,531	0,200
pH with H_2O_2	-2,838	-1,419	0,867	-1,636	0,177
pH with TiO ₂	-1,512	-0,756	0,804	-0,940	0,400
H_2O_2 with TiO ₂	-5,959	-2,980	0,804	-3,705	0,021
$r^2 = 0,95463$					

TABLE 6: Analysis of variance (ANOVA) of the predicted model for the photodegradation process in reduction of absorbance.

Variation source	Quadratic sum	Nº of degrees of freedom	Quadratic mean	F	<i>p</i> -value
рН	162,672	1	162,672	45,008	0,000
H_2O_2	60,555	1	60,555	16,755	0,005
H_2O_2 with TiO_2	34,957	1	34,957	9,672	0,017
Standard error	25,300	7	3,614		
Total	297,029	10			

Bacteria	CPX (Mean value of inhibition halo in mm)	CPX treated (Mean value of inhibition halo in mm)
E. coli	S (21.5)	R (14.0)
K. pneumoniae	S (22.0)	S (20.0)
A. hydrophila	S (28.0)	R (18.5)
S. aureus	S (38.5)	S (36.5)
S. xylosus	R (7.0)	R (7.0)

TABLE 7: Sensitivity of bacteria to CPX before and after irradiation by UV light.

R: resistant; S: sensitive; I: intermediate;

* lack of CPX activity; **Although not losing activity the decrease of halo was expressive

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147

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