CI&CEQ
Chemical Industry & Chemical Engineering Quarterly

Available online at
Association of the Chemical Engineers of Serbia *AChE*
www.ache.org.rs/CICEQ

Chem. Ind. Chem. Eng. Q. 28 (3) 179–190 (2022) CI&CEQ

ALEX LEANDRO ANDRADE DE LUCENA
RAYANY MAGALI DA ROCHA SANTANA
MARCOS ANDRÉ SOARES DE OLIVEIRA
LUCIANO COSTA ALMEIDA
MARTA MARIA MENEZES BEZERRA DUARTE
DANIELLA CARLA NAPOLEÃO

Chemical Engineering Department Federal University of Pernambuco, Recife, Brazil

SCIENTIFIC PAPER

UDC 615.2:502:66:543.544

EVALUATION OF COMBINED RADIATION FOR THE TREATMENT OF LAMIVUDINE AND ZIDOVUDINE VIA AOP

Article Highlights

- Use of advanced oxidation processes to degrade antiretrovirals applying combined irradiation
- Degradation of the pharmaceuticals lamivudine and zidovudine in a synthetic effluent
- Degradation of 90.53% for photoperoxidation and 89.32% for photo-Fenton in aqueous drug mixture
- Degradation of 88.69% for photoperoxidation and 85.79% for photo-Fenton in synthetic mixture
- Toxicity tests for the drugs, after submissions to the AOP, in both studied media

Abstract

The presence of pharmaceutical contaminants in nature is an environmental problem generating increasing concerns. Due to this, it is necessary to evaluate treatments capable of degrading these contaminants, such as the advanced oxidation processes (AOPs). In this work, the photoperoxidation and photo-Fenton AOP were applied to degrade a mixture of lamivudine and zidovudine in an aqueous medium and synthetic effluent (SE). To this end, a bench reactor (UV-C; UV-A and sunlight irradiations) was built. The AOP treatments efficiency was evaluated by ultraviolet/visible spectrophotometry. The tests involved the application of the irradiations individually and combined. The best operational conditions were [H_2O_2] of 600 mg L^{-1} and [Fe] of 0.5 mg L^{-1}, for both matrices, with degradations of 90.53% and 89.32% for the photoperoxidation and photo-Fenton processes in aqueous media and 88.69% and 85.79% in SE. Kinetic studies showed a good fit for two pseudo-first-order models with R^2 > 0.93. Toxicity tests involving the application of lettuce, carrot, and tomato seeds showed an inhibition for the three seeds when submitted to solutions after treatment, for both matrices, this fact is corroborated by the HPLC analysis, in which the formation of small peaks was verified, suggestive of the formation of by-products. Thus, it can be affirmed that both photo-Fenton and photoperoxidation processes efficiently degrade the drug mixture when applying UV-C radiation.

Keywords: chromatography, drugs, kinetic modeling, synthetic effluent, toxicity.

The pharmaceuticals comprise a large group of active medicinal products, used for personal health or aesthetics, to evoke biological or physiological responses in the target of use, indispensable for living beings' health [1,2]. Among the various classes of pharmaceuticals, products are the antiretrovirals, which are used to treat diseases such as influenza, herpes, hepatitis, and, in particular, the human immunodeficiency virus (HIV) [3,4]. Due to their efficiency in treating HIV, the use and application of antiretrovirals have grown rapidly worldwide, with many of them being considered essential by the world health organization (WHO) [5,6].

In the class of antiretrovirals, the following ones

Correspondence: D.C. Napoleão, Chemical Engineering Department Federal University of Pernambuco, Av. Prof. Arthur de Sá, s/n, 50740-521, Recife - PE - Brazil.
E-mail: daniella.napoleao@ufpe.br
Paper received: 9 March, 2021
Paper revised: 16 July, 2021
Paper accepted: 17 Avgust, 2021

https://doi.org/10.2298/CICEQ210309029L

stand out as the most commonly used: abacavir, nevirapine, stavudine, lamivudine (3TC), and zidovudine (AZT). The latter two are usually combined therapy to treat acquired immunodeficiency syndrome (AIDS) [7,8].

The increasing use of antiretrovirals drugs has generated an environmental concern. Without completely metabolizing the body, these substances are excreted via urine and feces, following the sewage collection network to the wastewater treatment plants (WWTPs) [3,8]. In these, the conventional physical-chemical and biological treatments are not efficient for completely degrading pharmaceutical contaminants, meaning that part of them can reach nature. This concern covers WWTPs related to domestic and industrial effluents [9].

As a consequence, there is an increasingly common identification and presence of drugs such as lamivudine and zidovudine in nature, with the majority of cases being identified in the African continent [8-12] and some in Europe [13-15], showing the scope of this type of contamination. This indicates that such substances may be present in countries that commonly use these drugs to treat diseases but have not yet identified or studied this type of problem in depth.

Knowing this, it is necessary to evaluate the efficiency of other treatments that act as an alternative or addition to the conventional processes, making it possible to degrade these contaminants efficiently [16,17] completely. The advanced oxidation processes (AOPs) are among alternative treatments, which can completely degrade organic contaminants, oxidizing them to non-toxic, smaller molecules or mineralizing to water, carbon dioxide, and mineral salts [18-20].

Different types of AOPs have been used to treat water and effluents, deserving of their investigation, capacity, and efficiency in the degradation of pharmaceuticals contaminants, the photoperoxidation, and photo-Fenton processes [8,21,22]. The photoperoxidation consists of a combination of solar or ultraviolet (UV) radiation with hydrogen peroxide (H_2O_2) to promote the formation of hydroxyl radicals (OH•). These are highly reactive and capable of degrading complex organic pollutants [23,24]. While the photo-Fenton process involves the reaction between (Fe^{2+}/H_2O_2) and (Fe^{3+}/H_2O_2) for the formation of hydroxyl radicals, in the presence of visible or UV radiation, with iron acting as a catalyst [25-26].

Several studies have suggested and proved the applicability of these two AOP as an effective tertiary treatment for the degradation of different classes of pharmaceutical contaminants [27-29]. These studies are important since drugs that resist the treatment present on WWTPs have been identified in other aquatic bodies. This fact can be evidenced by the average removal of lamivudine and zidovudine in wastewater treatment plants, which respectively reach values of the order of 76% and 68% [8,30].

When working with (AOPs)s to degrade different types of organic contaminants, some steps are essential: to evaluate the parameters involved, the controlling kinetics of the process, and analyze the toxicity [31]. The degradation kinetics of organic contaminants by AOP can be described, in general, by rate expressions of pseudo-first-order, as according to Eq. (1) [32,33]:

$$-\ln\frac{c}{c_0} = k \cdot t \quad (1)$$

where c is the concentration in a specific time, c_0 is the initial concentration, k is the rate constant of the system under study, and t is the reaction time.

The toxicity assessment is also important, considering that the intermediates and products formed in the degradation process can be more biologically active and, consequently, more toxic than the original compounds. Thus, evaluating the response of different organisms is crucial after applying AOPs as a treatment [34].

However, despite the above, studies on the removal of the pharmaceuticals lamivudine and zidovudine using AOP are still rare when compared to other classes of drugs [14,35]. Thus, this work aims to treat a mixture of the drugs lamivudine and zidovudine using the photoperoxidation and photo-Fenton processes, started by Lucena *et al.* [36], including a new analysis of the efficiency of the AOP not only in an aqueous medium but also, in synthetic effluent, besides evaluating the efficiency of the AOP when combined radiation is applied. To this end, kinetic studies were carried out to assess the suitability of the pseudo-first-order kinetic models to the concentration and degradation data of the drugs under investigation. Complementing the study, the efficacy of the process applied to the degradation of the drugs was investigated through a toxicity test using seeds of *Lactuca sativa* (lettuce), *Daucus carota* (carrot), and *Solanum lycopersicum* (tomato). Finally, chromatographic analysis evaluated the formation of by-products to understand better the results obtained after the submission of drugs to AOP treatments. The drugs and zidovudine are produced by the Laboratório Farmacêutico de Pernambuco (Pharmaceutical Laboratory of Pernambuco – LAFEPE), located near the Federal University of Pernambuco (UFPE), and a stream that is used as a receiving body for the effluents treated by both LAFEPE and UFPE. Allied to this, the

study by Funke *et al.* [14] indicates the resistance of these two drugs to conventional effluent treatments.

MATERIALS AND METHODS

Identification/quantification of the pharmaceuticals and preparation of the working solutions

The active principles of lamivudine (3TC) and zidovudine (AZT) were provided by LAPEFE with batch numbers 17933 and 17925, respectively. Both with a purity degree > 99%. The physicochemical properties of both drugs, such as molecular formula, weight, solubility in water, pK_a, and log K_{now}, are given in Supplementary Table 1.

A stock solution containing 1000 mg L^{-1} of the mixture of drugs 3TC and AZT was prepared using distilled water and methanol (Merck brand) in a volumetric ratio of 9:1. The work solutions used were prepared by diluting the stock solution in water. These solutions were analyzed using an ultraviolet/visible (UV/Vis) spectrophotometer (Thermoscientific) at a maximum absorbance wavelength (λ) of 269 nm. For quantification, was used, an analytical curve with a linear range from 1 to 30 mg L^{-1}, correlation coefficient > 0.99, variance coefficient of 1.43%, and limits of detection (LOD) and quantification (LOQ) equals 0.77 mg L^{-1} and 2.34 mg L^{-1}, respectively.

The synthetic effluent (SE) was composed of inorganic salts and other organic compounds, as described elsewhere [37, 38]. The components present in the SE and their respective concentrations are described in Table 1.

Table 1. Components and their concentrations in the synthetic effluent

Components	Concentration (mg·L^{-1})
Lamivudine	15
Zidovudine	15
Sucrose	25
Glucose	15
Urea	20
Ammonium phosphate	20
Sodium sulfate	25
Sodium chloride	25
Sodium carbonate	25
Sodium acetate	20
Magnesium carbonate	25
Potassium nitrate	10

Bench reactor configuration

The degradation tests were performed comparing the efficiency of the AOPs of photoperoxidation (UV/H_2O^{-2}) and photo-Fenton ($H_2O_2/Fe^{2+}/UV$) in addition to the photolysis process. For this purpose, a bench photolytic reactor equipped with lamps emitting three types of radiation (UV-C, UV-A, and Sunlight) was used, as shown in Figure 1.

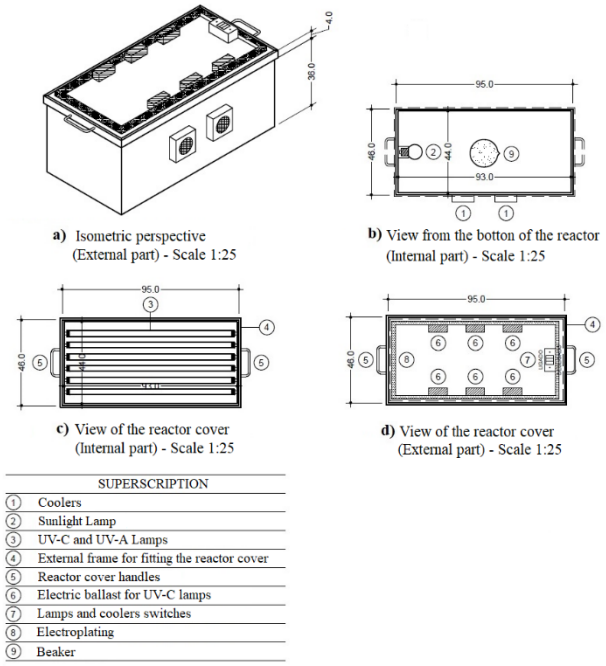

Figure 1. Bench-top photolytic reactor with combined radiation, general views, and isometric perspective. Measures in centimeters.

As can be seen in Figure 1, the reactor is equipped with three UV-C germicidal fluorescent tube lamps (Osram brand) of 30 W, three UV-A tube lamps (Tovalight brand) of 20 W, these two types installed interchangeably on the cover, and a 300 W sunlight lamp (Osram brand). When evaluated separately through radiometers (Emporionet), the radiations from these lamps had photon emission of respectively $3.32 \cdot 10^3$ W cm-^{2}, $1.39 \cdot 10^3$ W cm^{-2}, and $1.58 \cdot 10^5$ W cm^{-2}. In addition to the lamps, two coolers (Darkforce) were installed in the reactor to assist in cooling and temperature control. The photoreactor was coated internally with aluminum foil to increase its efficiency as according to Khan; Tahir [39].

Bench reactor cost analysis

A cost analysis for the assembly and operation of the reactor was also carried out. The costs were divided into 3 types: Project, materials, and operational. In the project costs, technical drawings were outlined, such as

general views and perspectives and the values associated with the construction of the reactor structure and the installation of lamps and electrical parts. The material costs were considered expenses with lamps, electrical ballasts, coolers, and electrical material such as wires and switches. At the same time, the operational cost was calculated based on the kW·h price of US$ 0.166 for B3 units of the classification from the Companhia Energética de Pernambuco/Brazil (CELPE), which includes the Federal University of Pernambuco, where the research was developed [40]. Thus, it was taken into account the operational time of the reactor and the power of the lamps and coolers installed.

Degradation of the drugs using AOPs

To perform the preliminary tests (in triplicate), beakers containing 50 mL of the aqueous mixture of drugs with initial concentrations of 15 mg L^{-1} were subjected to treatments for 60 min. In this step, a concentration of hydrogen peroxide ([H_2O_2]) (Exodus Cientifica brand) of 100 mg L^{-1} was used; an iron concentration ([Fe]) of 1 mg L^{-1} (present as de $FeSO_4$ $7H_2O$, F Maia Brand) and pH between 5 and 6 adjusted using solutions of H_2SO_4 and NaOH of 1 mol L^{-1} for the photolysis and photoperoxidation processes and between 2 and 3 for the photo-Fenton process. The efficiency of the AOP was determined based on the percentage of drug degradation after UV/Vis spectrophotometry analysis.

Based on the preliminary study results for each of the evaluated AOP, the influence of the operational parameters was assessed in more detail. For this purpose, experiments were carried out, varying the H_2O_2 concentration from 100 to 900 mg L^{-1}. Once the best [H_2O_2] was determined, it was fixed, and an analogous study was done for [Fe], between 0.5 and 5.0 mg L^{-1}. These tests were performed for an exposure period of 60 min for each irradiation (UV-C, UV-A and sunlight) individually.

After defining the influence of the variables [H_2O_2] and [Fe], degradation tests were performed to evaluate the effect of the combination of light irradiations on the efficiency of both AOP under study. For this, 50 ml solution of the drug mixture was used.

The types of combinations used took into account the operation of the lamps, in parallel and series, as shown in Figure 2. All tests exposed the working solution to 60 min for parallel experiments and 120 min for the tests in series, 60 min for each irradiation.

After defining the best operational conditions and evaluating the efficiency of each irradiation, individually and in a combined way, the exposure time of each process was extended to 90, 120, 150, and 180 min. Then, the distance from the light source to the photoreactor was evaluated in two situations: 1) 36 cm distance to the light source (Low Position) and 12 cm to the light source (High Position).

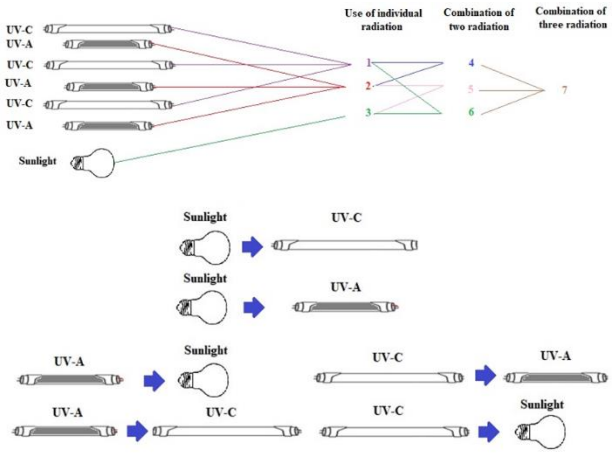

Figure 2. Application of combined irradiation, forms of organization.

Treatment of the synthetic effluent by AOP

Initially, the synthetic effluent (SE) degradation under study (described in item 2.1) was based on the best experimental conditions optimized for treating the drug aqueous mixture. With the SE being submitted to the photoperoxidation and photo-Fenton AOP. Bearing in mind that the presence of organic compounds and inorganic salts can affect the efficiency of the photo-Fenton process, during the experiments with the SE, the effect of the [Fe] was reevaluated in the same range used for the tests with the aqueous mixture.

The exposure times were 60, 90, 120, 150, and 180 min for both processes, with the study of [Fe] being conducted only for 60 min. Before and after being treated with the AOP, the samples were subjected to a UV-Vis spectrophotometry analysis.

Kinetic study and modeling

With the best experimental conditions, kinetic monitoring of the photoperoxidation and photo-Fenton AOP was performed, both in aqueous media and in the form of SE. For this purpose, 50 mL of each sample containing 15 mg L^{-1} of the pharmaceuticals were irradiated for 180 min, with regular aliquots being analyzed at 5, 10, 15, 20, 30, 45, 60, 75, 90, 120, 150, and 180 min. To not interfere with the analysis result, the aliquots read were returned to the container during the entire process. The experimental data were evaluated for suitability to the pseudo-first-order kinetic models [41,42]. The Chan and Chu model is described

by Eq. (2):

$$c = c_0\left(1-\frac{t}{\rho+\sigma t}\right) \quad (2)$$

where c is the pharmaceutical concentration ($mg\cdot L^{-1}$) after treatment by the AOP at a time t (min); c_0 is the initial concentration of the pharmaceuticals ($mg\cdot L^{-1}$), and the parameters 1/σ and 1/ρ symbolize, respectively, the oxidative capacity of the system (dimensionless) and the speed constant of the system (min^{-1}). The values for the parameters σ and ρ were found based on the linearization of Eq. (2), as expressed in Eq. (3):

$$\frac{t}{\left(1-\frac{c}{c_o}\right)} = \rho+\sigma t \quad (3)$$

Another model used to adjust the experimental data was the one proposed by He *et al.* [42], which is based on a simplification of the Langmuir-Hinshelwood model, Eq. (4), to adapt it to a pseudo-first-order equation generating Eq. (5).

$$-\frac{dc}{dt} = \frac{k_r\cdot K\cdot c}{1+K\cdot c} \quad (4)$$

$$-\frac{dc}{dt} = k_r\cdot K\cdot c = k\cdot c \quad (5)$$

where c is the pharmaceutical concentration ($mg\cdot L^{-1}$) and k is the pseudo-first-order reaction rate (min^{-1}).

Then, the degradation of the drugs and the formation of by-products were evaluated using a methodology of analysis by high-performance liquid chromatography, using an HPLC/UV (Shimadzu SS-550).

Analysis of the drugs lamivudine and zidovudine via HPLC/UV

For this purpose, analytical curves were built in the concentration range from 1 to 30 mg L^{-1} for both drugs, with detection and quantification limits, respectively of 0.36 mg L^{-1} and 1.10 mg L^{-1} for lamivudine and 0.41 mg L^{-1} and 1.25 mg L^{-1} for zidovudine. The chromatographic system consisted of a mobile phase with acetonitrile and water acidified with 0.1% acetic acid (65:35 v/v) and an ultra C18 Column (5 µm; 4.6 x 250 mm) operating in reverse with isocratic elution.

The equipment is composed of a UV detector, with analysis at 255 nm (zidovudine) and 277 nm (lamivudine). The system flow was fixed at 0.7 mL min^{-1}, with an oven temperature of 40 ± 1 °C and a pressure of 90 kg cm^{-2}. The injection volume used was equal to 5 µL, and the retention time varied between 5.0 min to 5.7 min for lamivudine and 5.8 min to 6.5 min for the zidovudine. To ensure the efficiency of the chromatographic analysis, the samples before and after submission to treatment via AOP were submitted to a preparation step using polymeric cartridges of the Strata X type (Phenomenex – 500 mg/6 mL) to perform a solid-liquid extraction (ESL). The operational mode was the reverse type, following the methodology described by Napoleão *et al.* [43]

Kinetic study and modeling

Toxicity tests were performed by applying seeds, using the following species *Lactuca sativa* (lettuce), *Daucus carota* (carrot), and *Solunum lycopersicum* (tomato). These were exposed to solutions with the pharmaceuticals contaminants before and after being treated by the photoperoxidation and photo-Fenton processes. The toxicity assessment followed the methodology described by Santos *et al.* [44].

Distilled water and a boric acid solution at 3% were used as negative and positive controls, respectively. Petri dishes were kept in an environment in the absence of light at 25±1 °C for a period of 120 h. Finally, the quantities of germinated seeds and the root growth were verified and used to calculate the relative growth index (RGI) and the germination index (GI) as according to Equations 6 and 7 [45].

$$RGI = \frac{CRA}{CRC} \quad (6)$$

$$GI = RGI\,\frac{SGA}{SGC} \quad (7)$$

where CRC is the total length of the root in the negative control, CRA is the total length of the root in the sample, SGC is the number of seeds germinated in the negative control, and SGA is the number of seeds germinated in the sample.

RESULTS AND DISCUSSION

Bench-top photolytic reactor with combined radiation – Associated costs

As mentioned in the methodology, the costs related to the construction and operation of the reactor were divided into 3 types, with the total cost of the reactor project estimated at US$120.00, US$40.00 related to the technical design, US$60.00 for the construction of the reactor structure and $20.00 for labor (installation and startup). The costs related to the acquisition of materials for the bench photolytic reactor are shown in Table 2.

The sum of the project and material costs totaled US$479.92 (Table 2). On the other hand, operating

costs were calculated individually for each lamp present, considering its operation per hour and the relative kWh value in Recife. The values associated with these costs are shown in Table 3.

Analyzing Table 3, the approximate operating cost, calculated, per hour of operation was US$0.07649, taking into account all the lamps and the two coolers in operation, that is, in the condition of maximum expenditure.

Table 2. The benchtop photolytic reactor's construction and operating costs with combined radiation

Material	Unitary value (US$)	Units	Total Value (US$)
Germicidal ultraviolet tubular lamp (UV-C) – Osram Puritec – 30 W	23.85	3	71.55
Black light fluorescent lamp (UV-A) – Starlux – 20 W	13.69	3	41,07
Sunlight lamp – Osram – 300 W	84.81	1	84.81
Electric ballast for fluorescent lamp 20 W	9.00	3	27.00
Electric ballast for fluorescent lamp 32W	13.14	3	39.42
Electric material	26.32	1	26.32
Wood to build the reactor - plywood, glue, screw, others	41.20	1	41.20
Cooler Fan – Dark Force	8.10	2	16.20
Utilities	12.35	1	12.35
Total Material Costs			359,92

* Values for January 2019.

Table 3. Operating costs for the benchtop protolithic reactor with combined radiation from March 2019 to April 2020

Item	Individual power (W·h)	Units	Power (kW·h)	Individual cost (US$/h)
Germicidal ultraviolet tubular lamp (UV-C) – Osram Puritec – 30 W	30	3	0.09	0.015
Black light fluorescent lamp (UV-A) – Starlux – 20 W	20	3	0.060	0.010
Sunlight lamp – Osram – 300 W	300	1	0.3	0.051
Cooler Fan – Dark Force	1,44	2	0.00288	0.00049
Germicidal ultraviolet tubular lamp (UV-C) – Osram Puritec – 30 W	30	3	0.09	0.015
Total				0.07649

Drugs treatment using AOPs – aqueous mixture

The preliminary tests indicated no degradation of the aqueous mixture of the drugs under study when using the photolysis process, applying the irradiations individually. A similar result was observed when using the AOP with UV-A and sunlight irradiations separately. On the other hand, the photoperoxidation and photo-Fenton processes associated with UV-C irradiation showed photodegradation results of 17.76 ± 0.21% and 8.27 ± 0.05%, respectively, after 60 min of treatment.

Thus, studies on the concentrations of hydrogen peroxide [H_2O_2] and iron [Fe] were conducted to increase the degradation capacity of AOPs using UV-C irradiation. The data obtained for the isolated study of these variables are shown in Figure 3.

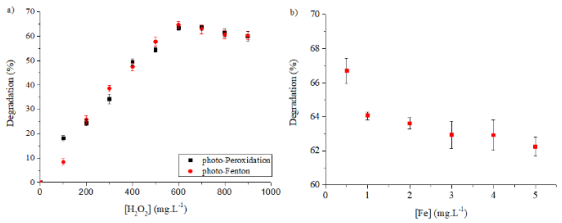

Figure 3. Influence of H_2O_2 and Fe concentrations on the degradation of the aqueous mixture of the drugs lamivudine and zidovudine by the photoperoxidation and photo-Fenton processes with 60 min of treatment, the study of the variation of a) [H_2O_2] ([Fe] = 0,5 mg·L^{-1}) and b) [Fe] ([H_2O_2] = 600 mg·L^{-1}).

From Figure 3a, it is observed that for [H_2O_2] above 600 mg L^{-1}, both studied processes lost

efficiency.This happens due to the non-selectivity of the H_2O_2 as a reagent and that when in excess, this oxidant can react in parallel with the hydroxyl radicals present in the solution. Thus, the formation of other radicals occurs, such as superoxide's, which are much weaker than hydroxyl; that is, they present lower potential reduction patterns [45,46]. Thus, it can be said that among the analyzed [H_2O_2], 600 mg L^{-1} is the limiting factor for both evaluated processes.

When evaluating Figure 3b, the increase in iron concentration does not improve the efficiency of the photo-Fenton process for degradation of the aqueous mixture of drugs 3TC and AZT. This figure clearly shows that [Fe] 0.5 mg L^{-1} led to further degradation. This indicates that the increase in iron in this process can act similarly to the excess of the oxidizing agent. Thus, there is an excessive release of hydroxyl radicals leading to the formation of other less desirable radicals, such as superoxide [47,48,49].

Then, the effect of applying the combined irradiation for both processes was evaluated. For these tests, degradation values in the range of 55 to 60% were verified only for combinations involving the presence of UV-C radiation, either in parallel or in series. So that there were no improvements in the yield of advanced oxidative processes for degradation of the mixture of drugs under study when applying the combined radiation. Thus, UV-C radiation individually was considered in all subsequent tests. Having determined the radiation to be employed, the effect of the distance from the irradiation source of the photocatalytic reactor to the mixture of drugs was evaluated. The results of this analysis are shown in Table 4.

Table 4. Effects of time and distance to light on the degradation of the aqueous mixture of lamivudine and zidovudine (15 mg·L[1])

Time (min)	Photoperoxidation		Photo-Fenton	
	Degradation (%)			
	Low position	High position	Low position	High position
60	62.73	78.40	66.69	83.43
90	78.46	84.82	78.88	85.59
120	86.70	88.63	86.71	86.55
150	88.56	89.66	86.97	88.18
180	90.13	90.53	87.58	89.32

*Low position – 36 cm of distance to the light source; High position – 12 cm of distance to the light source.

Table 4 shows that the HIGH position favors the yield of both studied AOP during the first 90 min of the process. However, after 180 min of treatment, there is no significant difference between the results obtained for the two positions, HIGH and LOW, with the degradation stabilizing in the range of 87 to 90%.

This behavior is explained by the design of photolytic reactors, aiming to ensure that all light emitted during the process remains inside the reactor. Therefore, the distance from the irradiation source (lamp) can be ignored. For this, reflective surfaces ensure that the real distance traveled by the light inside the reactor is greater than its physical dimensions [50]. In the present study, this observation was guaranteed by coating the reactor with aluminum foil.

Given the above and considering that the two positions studied stabilized in the same range of degradation in both processes, the amount of light absorbed by the organic substrate (drugs under the study) is similar, according to Crittenden *et al.* [50]. Thus, regardless of the position of the system in the reactor, there is no difference between the situations studied after stabilizing the process. This allows the use of the HIGH position as a standard for subsequent steps. Then, the efficiency of the photoperoxidation and photo-Fenton processes was evaluated to degrade the synthetic effluent.

Drugs treatment using AOPs – synthetic effluent

The treatment of the mixture of drugs in a medium consisting of synthetic effluent was carried out using the best experimental/operational conditions found for the aqueous mixture for both AOP. Therefore, 600 mg L^{-1} of [H_2O_2] and 0.5 mg L^{-1} of [Fe] were used, with the reactor fixed in the high position and the application of UV-C irradiation alone. The results obtained for these tests are shown in Table 5.

Table 5. Degradation of the synthetic effluent of the drugs lamivudine and zidovudine (15 mg·L^{-1} each) during 180 min of treatment under the photoperoxidation and photo-Fenton processes

Time (min)	Photoperoxidation	Photo-Fenton
	Degradation (%)	
60	64.96	81.49
90	71.25	83.09
120	84.57	84.42
150	86.06	84.99
180	88.69	85.79

Observing Table 5, it is clear that the mixture of drugs 3TC and AZT in synthetic effluent is more

to the photo-Fenton process in the first 90 min of treatment, with a difference of 11.84% in degradation when compared to the photoperoxidation process. However, both processes stabilize in the same range of degradation, between 85.79 and 88.69%. This fact was observed after 180 min of treatment, with no significant difference between both efficiencies.

The effect of [Fe] was reevaluated for the synthetic effluent, considering that the presence of inorganic salts and organic compounds in the studied matrix can impact the production of hydroxyl radicals according to Gil; Galeano; Vicente, [25]. The results for this analysis are shown in Figure 4.

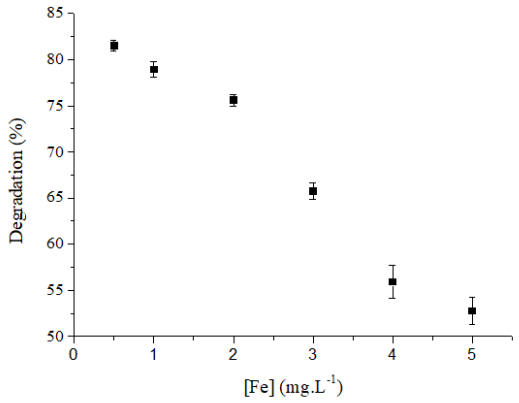

Figure 4. Influence of [Fe] on the degradation of a synthetic effluent containing lamivudine and zidovudine by the photo-Fenton process with 60 min of treatment

From Figure 4, it is evident that just like in the aqueous medium, the [Fe] that leads to the highest degradation efficiency was 0.5 mg L^{-1}. It is also possible to observe a more pronounced drop in the efficiency of the photo-Fenton by increasing the [Fe] in the treatment of the synthetic effluent. This fact must be related to the sum of the effects of excess iron with the action of inorganic salts and organic compounds, decreasing the production of hydroxyl radicals.

Kinetic study

Proving the efficiency of AOP for degradation of the mixture of drugs in both matrices studied, the reaction kinetics of these processes was evaluated. The kinetic study employed the following experimental conditions, for both processes [H_2O_2] of 600 mg L^{-1}, [Fe] of 0.5 mg L^{-1}, drug concentration of 15 mg L^{-1}, isolated application of UV-C irradiation, and the reactor in the HIGH operating position, with the kinetic monitoring performed for 180 min. From the data obtained in this study, the pseudo-first-order kinetic models developed by Chan and Chu [41] and He *et al.* [42] and the adequacy between them and the experimental data were evaluated. These results are shown in Figure 5.

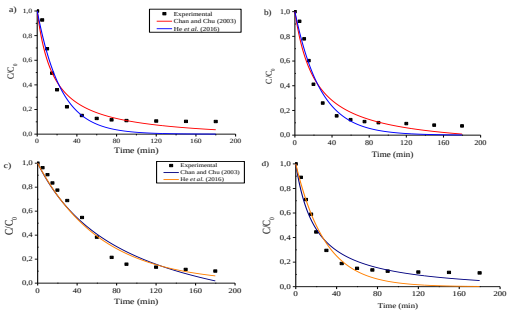

Figure 5. Adjustment to the models proposed by Chan and Chu [41] and He et al. [42] for the processes of a) photoperoxidation and b) photo-Fenton in aqueous medium and c) photoperoxidation and d) photo-Fenton in synthetic effluent. Conditions: [H_2O_2] = 600 mg·L^{-1}, [Fe] = 0,5 mg·L^{-1}, drug concentration = 15 mg·L^{-1}.

From Figure 5, it is observed that the degradation of the drugs in aqueous and synthetic media occurs more accentuated in the first 60 min of the process, stabilizing after 180 min, for both AOPs. The data of the obtained kinetic parameters are shown in Table 6.

Analyzing Table 6, it is observed that both models fit satisfactorily to the experimental data since the linear regression coefficients (R^2) are greater than 0.93. When comparing the models to each other, it can be seen that the model by Chan and Chu [41] presents a better fit in three of the four cases presented, with the model by He *et al.* [42] adapting better only to photoperoxidation in synthetic effluent.

There is also a higher rate of degradation (min^{-1}) for the photo-Fenton process in both matrices studied. This fact is evidenced by the greatest degradations obtained in the first 90 min of this treatment. On the other hand, there is no significant difference in the oxidative capacity between the studied processes, considering that both stabilize in a similar range of degradation % after 180 min of treatment.

Another way to demonstrate that the kinetic models of Chan and Chu [41] and He *et al.* [42] satisfactorily represent the degradation processes of the drugs lamivudine and zidovudine with the studied AOPs is to analyze the residual fraction of the drug mixture as a function of time and compare the data obtained experimentally with those predicted by the models, as shown in Figure 6.

The comparison between the experimental data

Table 6. Parameters for the kinetic models of Chan and Chu [41] and He et al. [42] for the degradation of the mixture of the drugs lamivudine and zidovudine by the photoperoxidation and photo-Fenton processes in aqueous and synthetic media

Process	Medium	Degradation (%) after 180 min	Chan and Chu [41] model			He *et al.* [42] model	
			$1/\rho$ (min^{-1})	$1/\sigma$	R^2	K (min^{-1})	R^2
Photoperoxidation	aqueous	92.5	0.077	1.007	0.95	0.043	0.93
Photo-Fenton		89.8	0.088	0.972	0.95	0.052	0.94
Photoperoxidation	synthetic	89.9	0.022	1.090	0.94	0.016	0.97
Photo-Fenton		88.8	0.050	1.029	0.98	0.035	0.97

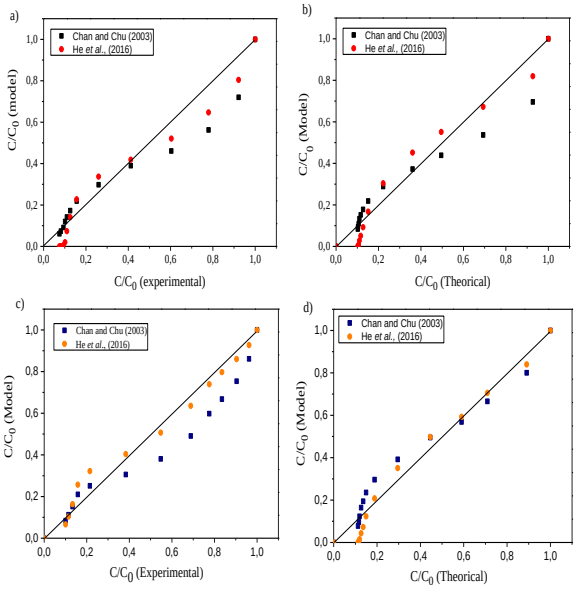

Figure 6. Comparison of the values of experimental residual concentration and the ones predicted by the models of Chan and Chu [41] and He et al. [42] for the processes of a) photoperoxidation and b) photo-Fenton in aqueous medium and c) photoperoxidation and d) photo-Fenton in synthetic effluent. Conditions: [H_2O_2] = 600 mg·L^{-1}, [Fe] = 0,5 mg·L^{-1}, drug concentration = 15 mg·L^{-1}.

and the models shown in Figure 6 confirms that the pseudo-first-order kinetic models proposed by Chan and Chu [41] and He *et al.* [42] satisfactorily describe the experimental results found in the treatment of drugs by AOPs in both aqueous media and in the synthetic effluent. Bearing in mind that the closer the experimental values are to the first bisector shown in the graphs, the better their help to the proposed models. This indicates that the trend of degradation for the lamivudine and zidovudine drugs follows the expected behavior for the treatment of pharmaceuticals via AOPs, that is, a fast first step (60 min) responsible for the degradation of 90.31% of lamivudine and 85.31% of zidovudine under the photoperoxidation process and 87.67% of lamivudine and 85.93% of zidovudine under the photo-Fenton process, followed by a slow step, which for this study stabilized at 180 min.

The samples were then evaluated on the HPLC to verify the degradation of both drugs under AOP treatment in the two matrices applied, aqueous mixtures and synthetic effluent. The chromatograms for these analyzes are shown in Figure 7.

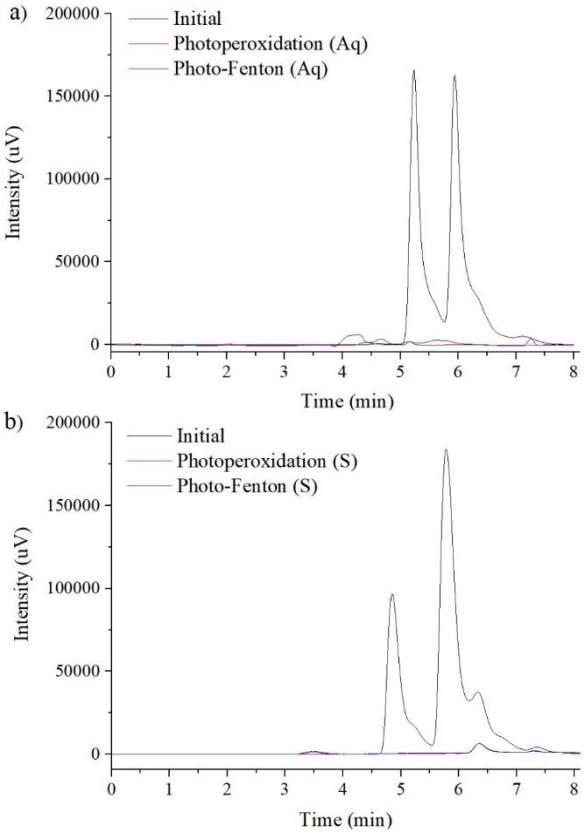

Figure 7. Chromatograms before and after applying the photoperoxidation and photo-Fenton processes for a) the aqueous solution of lamivudine and zidovudine and b) synthetic effluent.

From Figure 7, it was verified that both treatments (photoperoxidation and photo- Fenton) completely degrade the studied drugs on both matrices. However, it is possible to observe the presence and formation of three small peaks in the

retention times of 4.0 to 4.4 min, 4.5 to 4.7 min, and from 7.1 to 7.4 min after the photo-Fenton treatment. While for the synthetic effluent analysis, two small peaks were identified, at the retention times of 3.2 to 3.8 min, for the photoperoxidation processes and from 6.3 to 6.6 min for both AOP processes.

After evaluating the degradation efficiency for the two pharmaceuticals studied, lamivudine and zidovudine, in both applied matrices, a residual peroxide analysis was carried out following the methodology described by Santana *et al.* [51], with results ranging from 30 mg L^{-1} to 60 mg L^{-1} for all treatment processes applied.

Although a high degradation efficiency was obtained for the drug mixture under study, it is known that by treating organic pollutants via AOPs, by-products can be formed during the treatment. Thus, based on literature data [52,53], it is possible to propose the degradation kinetics for the mixture of drugs (Lamivudine - $C_8H_{11}N_3O_3S$ and Zidovudine - $C_{10}H_{13}N_5O_4$) under study, with its probable intermediates, as can be seen in Equation 8.

The presence of by-products and intermediates is an important factor that can affect and influence the toxicity effect of solutions under AOP treatments to different types of organisms. Thus, the application of more detailed assays to evaluate this possible toxicity is required when analyzing the availability of AOPs.

$$C_8H_{11}N_3O_3S + C_{10}H_{13}N_5O_4 + H_2O_2 \xrightarrow{Fe\ and/or\ hv} C_5H_7N_2O_2 + C_6H_{10}NO_3 + C_4H_6N_3O + C_4H_8O_2S \xrightarrow{HO^*} CO_2 + H_2O + HNO_3 \quad (8)$$

Toxicity

The toxicity tests carried out with seeds of the species *Lactuca sativa* (lettuce), *Daucus carota* (carrot), and *Solanum lycopersicum* (tomato) demonstrated that the application of the positive control resulted in the absence of germination. Data related to germination and root growth for negative control and test solutions (before and after treatment) are shown in Figure 8.

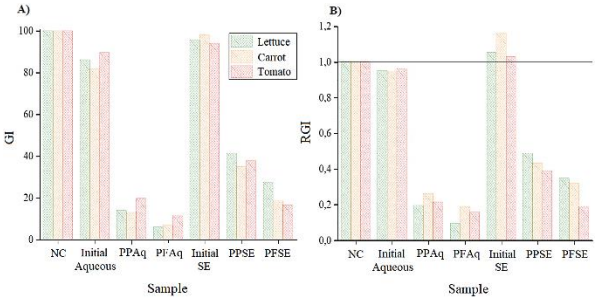

*Figure 8. A) Germination index (GI) and B) Relative growth index (ICR) for the seeds of Lactuca sativa (lettuce), Daucus carota (carrot), and Solanum lycopersicum (tomato). *NC - Negative control; PP_{Aq} - Photoperoxidation aqueous; PF_{Aq} - Photo-Fenton aqueous; PPSE - Photoperoxidation synthetic effluent; PFSE - Photo-Fenton synthetic effluent.*

Figures 8a and b show that the values of GI and RGI obtained for the tests in aqueous and synthetic media show some differences. The values obtained in the latter are higher than their equivalent in aqueous media for all samples analyzed. This behavior can be explained by the richness of nutrients in the prepared synthetic effluent, especially nitrogen, such as potassium nitrate, ammonium phosphate, and urea, an important element of the plant growth cycle in most terrestrial ecosystems [54,55].

The RGI values obtained for the photoperoxidation and photo-Fenton processes in both media studied were lower than 0.8 (less than 80% of the relative values for the negative control), indicating inhibition of seed growth according to Young *et al.* [45]. Such behavior was observed for the three seeds studied but not in the initial solutions, which indicated that the intermediates and product formed during the degradation processes might present greater toxicity than the original/initial pharmaceuticals contaminants. This is not unusual behavior. Hillis *et al.* [56] and Papaioannou *et al.* [35] also evaluated the toxicity of pharmaceuticals contaminants against seeds, not finding significant changes in germination and root growth of different species when in contact with solutions without treatment. These results show the need to improve the use of AOPs to obtain a further degraded and non-toxic final solution. Furthermore, it is of fundamental importance to test different organisms, as mentioned by Wang and Wang [57], as some may be more sensitive to certain types of pollutants and by-products formed.

CONCLUSION

The use of AOP to degrade a mixture of the drugs lamivudine and zidovudine presented the best results in the exclusive presence of UV-C radiation. The combined radiation was not significant for increasing under the optimized operational parameters, the degradation efficiency. After 180 min of treatment, degradations of 90.53% and 89.32%, respectively, for the photoperoxidation and photo-Fenton processes in aqueous medium and 88.69% and 85.79% for the same

processes in synthetic effluent were obtained. The models proposed by Chan and Chu [41] and He *et al.* [42] adjusted appropriately to the concentration data for drugs mixtures, R^2 values greater than 0.93, also, for both matrices, the photo-Fenton process had a higher rate of degradation, with greater decays in the first 90 min of treatment when compared to the photoperoxidation. From ecotoxicity studies with seeds, growth inhibition was verified for the solutions submitted to both processes and matrices, indicating an inhibition of the root growth of the evaluated species. This result also indicates that the intermediates formed during the degradation processes, which were confirmed by the HPLC analysis, may be more toxic than the initial solutions, a behavior similar to that found by some other works in the literature. The results obtained in this work show the efficacy and applicability of the AOPs to degrade a mixture of pharmaceuticals commonly used to control the acquired immunodeficiency syndrome (AIDS), with a satisfactory performance under both matrices applied. But new research is still required to further determine this application, especially in guaranteeing that the final products and by-products formed during those processes are safe and non-toxic to the environment.

Acknowledgment

To CAPES, FADE/UFPE, NUQAAPE/FACEPE (APQ-0346-1.06/14 process) and to Laboratório Farmacêutico de Pernambuco (Pharmaceutical Laboratory of Pernambuco - LAFEPE).

REFERENCES

[1] K. Balakrishna, A. Rath, Y. Praveenkumarreddy, K.S. Guruge, B. Subedi, Ecotoxicol. Environ. Saf. 137 (2017) 113-120.

[2] T. Mackulak, S. Cernanský, M. Fehér, L. Birosová, M. Gál, Curr. Opin. Environ. Sci. Health 9 (2019) 40-48.

[3] H. Zhou, T. Ying, X. Wang, J. Liu, Sci. Rep. 6 (2016) 1-10.

[4] S. Ncube, L.M. Madikizela, L. Chimula, M.M. Nindi, Water Res. 145 (2018) 231-247.

[5] J. Rwagitinywa, A. Sommet, A. Palmaro, J.L. Montastruc, M. Lapeyre-Mestre, Health Policy 122 (2018) 237-242.

[6] World Health Organization (WHO) - Model List of Essential Medicines 21st List (2019). p. 19.

[7] G. Kumari, R.K. Singh, HIV AIDS Review 11 (2012) 5-14.

[8] D. Russo, A. Siciliano, M. Guida, R. Andreozzi, N.M. Reis, G.L. Puma, R. Marotta, J. Hazard. Mater. 349 (2018) 195-204.

[9] L.M. Madikizela, S. Ncube, L. Chimuka, J. Environ. Manage. 253 (2020) 1-11.

[10] C. Schoeman, M. Dlamini, O.J. Okonkwo, Emerging Contam. 3 (2017) 95-106.

[11] T.T. Mosekiemang, M.A. Stander, A. Villiers, Chemosphere 220 (2019) 983-992.

[12] K.O. K'oreje, M. Okoth, H.V. Langenhove, K. Demeestere, J. Environ. Manage. 254 (2020) 109752.

[13] L. Vergeynst, A. Haeck, P.D. Wispelaere, H.V. Langenhove, K. Demeestere, Chemosphere 119 (2015) 52-58.

[14] J. Funke, C. Prasse, T.A. Ternes, Water Res. 98 (2016) 75-83.

[15] L. Boulard, G. Dierkes, T. Ternes, J. Chromatogr. A 1535 (2018) 27-43.

[16] A. Szymonik, J. Lach, K. Malinska, Ecol. Chem. Eng. 24 (2017) 65-85.

[17] K. Manoli, L.M. Morrison, M.W. Sumarah, G. Nakhla, A.K. Ray, V.K. Sharma, Water Res. 148 (2019) 272-280.

[18] A. Mirzaei, Z. Chen, F. Haghigthat, L. Yerushalmi, Chemosphere 174 (2017) 665-688.

[19] S. Ahuja, in Advances in Water Purification Techniques: Meeting the needs of developed and developing countries, Elsevier Inc. 1st Ed, Netherlands (2019). p. 135-156.

[20] J. Rodríguez-Chueca, S.V.D. Giustina, J. Rocha, T. Fernandes, C. Pablos, A. Encinas, D. Barcelo, S. Rodrígue-Mozaz, C. M. Manaia, J. Marugán, Sci. Total Environ. 652 (2019) 1051-1061.

[21] G. Lofrano, R. Pedrazzani, G. Libralato, M. Carotenuto, Curr. Org. Chem. 21 (2017) 1-14.

[22] K. Kowalska, G. Maniakova, M. Carotenuto, O. Sacco, V. Vaiano, G. Lofrano, L. Rizzo, Chemosphere 238 (2020) 1-8.

[23] D. Kanakaraju, B.D. Glass, M. Oelgemoller, J. Environ. Manage. 219 (2018) 189-207.

[24] S. Ameta, R. Ameta, in Advanced Oxidation Processes for Wastewater Treatment, Academic Press 1st Ed., Elsevier Inc. London (2018) p. 13-46.

[25] A. Gil, L.A. Galeano, M.A. Vicente, in Applications of Advanced Oxidation Processes (AOPs) in Drinking Water Treatment, Vol. 67. Springer. Cham, Switzerland (2019). p. 53-68.

[26] L. Ling, Y. Liu, D. Pan, W. Lyu, X. Xu, X. Xiang, M. Lyu, L. Zhu, Chem. Eng. J. 381 (2020) 122607.

[27] Y. Yang, Y. Cao, J. Jiang, L. Xinglin, J. Ma, S. Pang, J. Li, Y. Liu, Y. Zhou, C. Guan, Water Res. 149 (2019) 543-552.

[28] W. Qiu, M. Zheng, J. Sun, Y. Tian, M. Fang, Y. Fang, T. Zhang, C. Zheng, Sci. Total Environ. 651 (2019) 1457-1468.

[29] L. Ioannou-Ttofa, S. Raj, H. Prakash, D. Fatta-Kassinos, Chem. Eng. J. 355 (2019) 91-102.

[30] C. Prasse, M.P. Schlusener, R. Schulz, T.A. Ternes, Environ. Sci. Technol. 44 (2010) 1728-1735.

[31] M.I. Stefan, in Advanced Oxidation Processes for Wastewater Treatment - Fundamentals and Applications, IWA Publishing 1st Ed., London (2018) p. 39.

[32] Z. Khuzwayo, E.M.N. Chirwa, S. Afr. J. Chem. Eng. 23 (2017) 17-25.

[33] D.B. Miklos, C. Remy, M. Jekel, K.G. Linden, J.E. Drewes, U. Hubner, Water Res. 139 (2018) 118-131.

[34] A. Sharma, J. Ahmad, S.J.S. Flora, Environ. Res. 167 (2018) 223-233.

[35] D. Papaioannou, P.H. Koukoulakis, M. Papageorgiou, D.A. Lambropoulou, I.K. Kalavrouziotis, Chemosphere 238 (2020) 1-6.

[36] A.L.A. Lucena, D.C. Napoleão, H.V.C. da Silva, R.M.R. Santana, B.G. Ribeiro, M.M.M.B. Duarte, CeN. 42 (2020) e9

[37] A. Takdastan, A.H. Mahvi, E.C. Lima, M. Shirmardi, A.A. Babaei, G. Goudarzi, A. Neisi, M.H. Farsani, M. Vosoughu, Water Sci. Technol. 74 (2016) 2349-2363.

[38] A.G.N. Wamba, K.N. Sylvere, E.C. Lima, J.G. Kayem, P.S. Thue, T.M.H. Costa, A.B. Quevedo, E.V. Benvenutti, F.M. Machado, J. Taiwan Inst. Chem. Eng. 102 (2019) 321-329.

[39] A.A. Khan, M. Tahir, J. CO_2. Util. 29 (2019) 205-239.

[40] Companhia Energética de Pernambuco – CELPE. Tabela de Tarifas de Energia Elétrica. Grupo B. Resolução homologatória Nº 2535 de 25 de abril de 2019. Vigência: 29/04/2019 a 28/04/2020, 2019.

[41] K.H. Chan, W. Chu, Chemosphere 51 (2003) 305-311.

[42] Y. He, N.B. Sutton, H.H.H. Rijnaarts, A.A.M. Langenhoff, Appl. Catal. B 182 (2016) 132-141.

[43] D.C. Napoleão, L.E.M.C Zaidan, J.M. Rodríguez-Díaz, R.M.R. Santana, M.C.B.S.M. Montenegro, A.N. Araújo, M. Benachour, V.L. Silva, Afinidad. 75 (2018) 23-31.

[44] M.M.M. Santos, M.M.M.B. Duarte, G.E. Nascimento, N.B.G.D. Souza, O.R.S. Rocha, Environ. Technol. 40 (2019) 1494-1507.

[45] B.J. Young, N.I. Riera, M.E. Beily, P.A. Bres, D.C. Crespo, A.E. Ronco, Ecotoxicol. Environ. Saf. 76 (2012) 182-186.

[46] K.E.A. Ibrahim, D.J. Solpan, Environ. Chem. Eng. 7 (2019) 102820.

[47] N. López-Vinent, A. Cruz-Alcalde, L.E. Romero, M.E. Chávez, P. Marco, J. Giménez, S. Esplugas, J. Hazard. Mater. 380 (2019) 120882.

[48] E.C. Lumbaque, B.A. Silva, F.C. Bock, G.A. Helfer, M.F. Ferrão, C. Sirtori, J. Hazard. Mater. 378 (2019) 1-9.

[49] Y. Lan, L. Barthe, A. Azais, C. Causserand, Sep. Purif. Technol. 231 (2020) 1-10.

[50] J.C. Crittenden, R.R. Trussel, D.W. Hand, K.J. Howe, G. Tchobanoglous, in MWH' s Water Treatment: Principles and Design. 3rd Ed., Jonh Wiley & Sons, Inc. New Jersey (2012) p. 509-532.

[51] R.M.R. Santana, D.C. Napoleão, S.G.S. Júnior, R.K.M. Gomes, N.F.S. Moraes, L.E.M.C. Zaidan, D.R.M. Elihimas, G.E. Nascimento, M.M.M.B. Duarte, Chem. 75 (2021) p. 2305-2316.

[52] G. Bedse, V. Kumar, S. Singh, J. Pharm. Biomed Anal. 49 (2009) 55-63.

[53] M. Kurmi, A. Sahu, S. Singh, J. Pharm. Biomed. Anal. 134 (2017) 372-384.

[54] X. Xie, Q. Zhou, Z. He, Y. Bao, Environ. Toxicol. Chem. 29 (2010) 922-928.

[55] D. Sun, V. Mullerová, M.M. Ardestani, J. Froutz, Soil Tillage Res. 195 (2019) 1-9.

[56] D.G. Hillis, J. Fletcher, K.R. Solomon, P.K. Sibley, Arch. Environ. Contam. Toxicol. 60 (2011) 220-232.

[57] J. Wang, S. Wang, J. Clean. Prod. 315 (2021) 128202.

ALEX LEANDRO ANDRADE DE LUCENA

RAYANY MAGALI DA ROCHA SANTANA

MARCOS ANDRÉ SOARES DE OLIVEIRA

LUCIANO COSTA ALMEIDA

MARTA MARIA MENEZES BEZERRA DUARTE

DANIELLA CARLA NAPOLEÃO

Chemical Engineering Department Federal University of Pernambuco, Recife, Brazil

NAUČNI RAD

UTICAJ KOMBINOVANOG ZRAČENJA NA UKLANJANJE LAMIVUDINA I ZIDOVUDINA NAPREDNIM OKSIDACIONIM PROCESIMA

Prisustvo farmaceutskih zagađivača u prirodi je ekološki problem koji izaziva sve veću zabrinutost. Zbog toga je neophodno proceniti tretmane koji mogu da razgrađuju ove zagađivače, kao što su napredni oksidacioni procesi (AOP). U ovom radu, smeša lekova lamivudina i zidovudine je treitana u vodenoj sredini i sintetičkim rastvorima fotoperoksidacijom i foto-Fentonovim procesom. U ovu svrhu je korišćen stoni reaktor (UV-C, UV-A i zračenje sunčevom svetlošću). Efikasnost AOP tretmana je procenjena UV spektrofotometrijom. Testovi su uključivali primenu zračenja pojedinačno i kombinovano. Najbolji radni uslovi bili su [H_2O_2] od 600 mg/l i [Fe] od 0,5 mg/l, za obe matrice, sa degradacijom fotoperoksidacijom i foto-Fentonovim procesem, redom, od 90,53% i 89,32% u vodenim medijima. odnosno 88,69% i 85,79% u sintetičkim rastvorima . Istraživanja kinetike su potvrdila dva modela pseudo-prvog reda sa $R^2 > 0,93$. Testovi toksičnosti, koji su uključivali primenu semena zelene salate, šargarepe i paradajza, pokazali su inhibiciju za sva tri semena kada su podvrguna rastvorima nakon tretmana, za obe matrice Ovu činjenicu potvrđuje HPLC analiza, u kojoj je verifikovano formiranje malih pikova, koji ukazuju na formiranje sporednih proizvoda. Dakle, može se potvrditi da su procesi foto-Fentona i fotoperoksidacije efikasni u degradaciji smeše lekova kada se primenjuje UV-C zračenje.

Ključne reči: hromatografija, lekovi, kinetičko modelovanje, sintetički efluent, toksičnost.