Methotrexate Degradation by UV-C and UV-C/TiO$_2$ Processes with and without H$_2$O$_2$ addition on Pilot Reactors

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Abstract—Methotrexate (MTX) is an anti-cancer drug that can be excreted up to 90% after administration due to its low biodegradability. Advanced Oxidation Processes (AOPs) are a feasible alternative for the elimination of MTX in the environment. In this research, AOPs were performed in specialized patented reactors (UBE Photocatalytic systems and BrightWater Titanium Advanced Oxidation Process) under experimental pilot conditions. Photolysis and heterogeneous photocatalysis (UV and UV/TiO$_2$) experiments were performed with and without addition of H$_2$O$_2$ and at different initial pHs. Best degradation percentage was achieved by photolysis when initial pH was 3.5 and added H$_2$O$_2$ was 3 mM, reaching a MTX degradation of 82% after 120 min of reaction. HPLC-MS analysis of the resulting samples showed four possible byproducts of MTX degradation, which presented a higher ecotoxicity than the starting compound.

Index Terms—Methotrexate, photodegradation, water treatment, cytostatics.

I. INTRODUCTION

Emergent contaminants are natural or synthetic chemical compounds that are not included yet in international regulation but can nevertheless cause damage, especially when they have been released into the environment for long time periods as detection methods are not fully develop yet [1]-[3].

Emergent contaminants are categorized based on their application and are grouped in hormones, anti-inflammatories, antibiotics, antiepileptics, cytostatics, and beta blockers, among others [2], [3]. The way they are introduced into the environment depends upon their uses [4].

Methotrexate (MTX) is an antimetabolite and antifolate cytostatic drug, analog of folic acid, with mutagenic and teratogenic properties. It is widely used in high doses in chemotherapy to fight against various types of cancer, and in low doses for the treatment of rheumatoid arthritis, juvenile idiopathic arthritis, adult psoriasis, Crohn's disease and other diseases [5]-[8].

MTX is a highly polar and bioaccumulable compound as a consequence of being poorly metabolized once consumed. Twenty four hours after intake, up to 90% of the medication can be excreted through urine and feces without change. Therefore, it enters the hydrological cycle through urban and hospital wastewater and, according to some reports, it can be detected in drinking water [5]-[7].

Advanced Oxidation Processes (AOPs) represent an alternative technology for the degradation of recalcitrant organic compounds in wastewater [9], [10]. Such compounds present high chemical stability and/or low biodegradability, making them difficult to remove by conventional methods [9]-[11].

AOPs can be defined as chemical processes that involve the *in situ* generation of hydroxyl radicals ($\cdot$OH, e° = 2.8 eV) through various chemical, photochemical, sonochemical or electrochemical reactions [12]-[14]. Among AOPs, heterogeneous photocatalysis has attracted great attention in the last decade [15], being considered as one of the most attractive options for wastewater treatment due to its great potential and high efficiency [16], [17].

This document presents the results of the application of photolytic and photocatalytic processes to the degradation of methotrexate at three different pHs (acidic, basic and neutral) and compares the results when H$_2$O$_2$ is added as an oxidant in the processes. It also analyses the possible degradation byproducts and their toxicity when dissolved in water.

II. MATERIALS AND METHODS

Samples were produced by dissolving commercial drug MTX (Trixilem®, Lemery S.A. de C.V., Mexico) in distilled water until desired concentration. In UV-C experiments, a sample volume of 50 L was used, meanwhile UV-C/TiO$_2$ required a volume of 25 L, both with 5 mg/L MTX initial concentration.

A. UV-C and UV-C/H$_2$O$_2$ MTX Degradation Processes

UV reactor used for UV-C and UV-C/H$_2$O$_2$ experiments is shown in Fig. 1. The reactor system consists of a 200 L tank for the water sample, a 1 hp bomb for sample recirculation through the system, after the bomb, a 50 µm filter to prevent large particles from entering the reactor body, and a rotameter in order to measure the water flow going into the reactor. The reactor, main part of the system,

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is a compartment of stainless steel with the inlet in the bottom part and the outlet in the top. A low-pressure mercury lamp (254 nm radiation peak; T5, Philips) was used as irradiation source. Such lamp was placed inside a transparent quartz tube to prevent it from entering in contact with the sample. The tube is introduced by the top end of the reactor and occupies its center, so the radiation hits the polished reactor internal surface and reflects back to the sample [18], [19].

UV-C and UV-C/H_2O_2 experiments were performed at 650 L/h flow rate. Once sample recirculation started in the system, pH was adjusted with 65% (v/v) HNO_3 or 0.1 M NaOH solutions, to tested pHs: 3.5, 7.0 and 9.5. The effect of hydrogen peroxide on these MTX degradation processes was also tested; in this case, 3 mM H_2O_2 (Labkem, Spain, CAS: 7722-84-1) was added in half of the experiments, to be compared with the other half, carried out without added oxidant. After pH adjustment and H_2O_2 addition (when required), the first water sample was taken (time 0) and lamp was turned on in order to start the process. Aliquot samples were taken at 5, 10, 15, 20, 30, 45, 60, 90, and 120 min of reaction in order to perform the analysis and quantify MTX degradation.
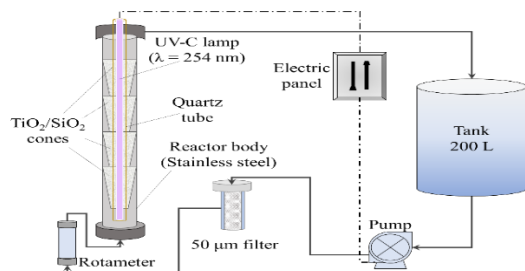

Fig. 1. Reactor system used for UV-C and UV-C/H_2O_2 MTX degradation processes.

B. UV-C/TiO₂ and UV-C/TiO₂/H₂O₂ MTX degradation processes

For photocatalysis experiments, the commercial AOP1 model reactor (BrightWater Environmental Ltd., UK) was used. This reactor is composed by a titanium cylinder covered in the internal wall by a titanium dioxide layer. Radiation is provided by a lamp emitting radiation at 254 nm and sample recirculation is achieved by a 0.85 kW bomb (Jet Inox 850). Fig. 2 shows the reactor, 75 mm diameter and 475 mm long. A flow rate of 500 L/h was set.

Excepting flow rate, photocatalysis experiments were conducted in the same conditions and procedure than UV-C and UV-C/H_2O_2 experiments: same tested pHs (3.5, 7.0, and 9.5), and added H_2O_2 (0 and 3 mM).
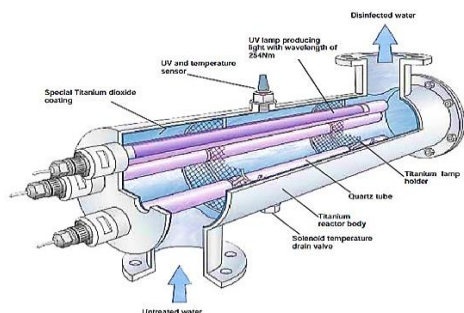

Fig. 2. Commercial photocatalytic UV reactor used in UV-C/TiO_2 and UV-C/TiO_2/H_2O_2 MTX degradation processes. The diagram is just representative, AOP1 model uses one lamp.

C. Chemical Analysis

1) MTX analysis

Water samples taken at initial time (0 min) and during the experiment were analyzed by UV/VIS spectrophotometry (T80+ UV/VIS Spectrophotometer, PG Instruments Ltd) at 303 nm wavelength to measure MTX concentration. Calibration line (R^2 = 0.9996) was built using MTX purchased from Sigma-Aldrich (CAS: 59-05-2, USA) dissolved in deionized water.

2) Ecotoxicity measurement

Water samples taken at 0, 60 and 120 min reaction times were tested for ecotoxicity in order to measure and compare potential environmental damage by MTX and its photodegradation byproducts. Such analyses were performed by the method UNE-EN ISO 11348-3 [20], which measures the effect of the sample on *Vibrio fischeri* luminescence. NaCl used for the method was purchased from Panreac (CAS: 7647-14-5, Spain) and bacteria was part of the commercial kit WATERTOX™ (Environmental Bio-detection Products Inc., Canada).

3) Residual H₂O₂ analysis

Residual H_2O_2 concentration was measured in samples taken at 5, 30, 60, and 120 min reaction times. Quantitative analysis was performed following the method previously used by Klamerth [21], which is based in the formation of a yellow solution when H_2O_2 and titanium oxysulfate are mixed, which absorbance is measured at 410 nm wavelength. Calibration line was built using H_2O_2 solutions with concentration ranging from 0.5 up to 3 mM. Titanium oxysulfate was purchased from Sigma-Aldrich (CAS: 13825-74-6, USA).

4) MTX byproducts formation

Water samples from experiments were analyzed by mass spectrometry in order to determine the possible chemical structures of MTX degradation byproducts. For comparison purposes, samples from times 0 and 120 min reaction times were analyzed by an Agilent 1100 HPLC coupled to an UV detector and a mass spectrometer Agilent Trap XCT and a HPLC Surveyor MS with a LTQ spectrometer.

D. Kinetic Analysis

Analysis of MTX concentration at different times of the reaction (section II.C.1) were used to determine reaction kinetic constants (k) by plotting ln[MTX] against time in the first 30 min range of reaction [22]. Once k was determined, half-life time ($\tau = \ln2/k$) of MTX degradation of each process was calculated.

III. RESULTS AND DISCUSSION

A. UV-C and UV-C/H₂O₂ MTX Degradation Experiments

There is an obvious difference from processes with and without addition of H_2O_2. According to Lutterbeck [6], at neutral pH close to 7, complete degradation of 20 mg/L MTX is reached after 128 min of UV irradiation, but with low mineralization. As in the present work the MTX degradation reached through UV irradiation was lower than 20% at the three tested pHs, data are not shown. Even

though direct photolysis of molecules present in wastewater is possible when irradiating with 254 nm wavelength, photolysis presents limitations and low efficiencies [23]. Besides, superoxide radical ($O_2^{\cdot-}$), formed in the presence of oxygen, is able to degrade substituted aromatic compounds with high absorption in the UV range but has a low oxidizing power [24].

MTX degradation was highly enhanced by the addition of hydrogen peroxide. As H_2O_2 is a weak acid with a pK_a of 11.6 [24], the effect of sample pH was of great importance. Fig. 3 shows MTX degradation by UV-C/H_2O_2 experiments where best results (82% MTX degradation), were achieved at initial pH of 3.5.
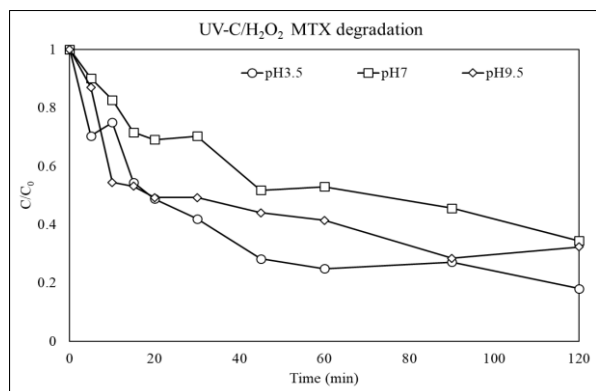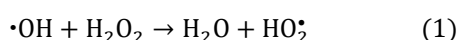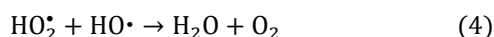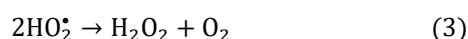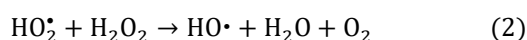

Fig. 3. MTX degradation in the UV-C/H_2O_2 processes at three different initial pHs.

When irradiated with radiation below 280 nm wavelength, O-O bonds of H_2O_2 break into HO• [25], thus degradation enhancement is to be expected. Nevertheless, best results should appear at alkaline conditions experiments given that the conjugate anion of H_2O_2 increases with pH and favors HO• production [24]. However, the best results were achieved at acidic pH, being a possible explanation the formation of less reactive $HO_2^{\cdot}$ radicals in •OH excess, through (1) [23]-[26]:

$$\cdot OH + H_2O_2 \rightarrow H_2O + HO_2^{\cdot} \tag{1}$$

$HO_2^{\cdot}$ radicals can later react as follows [27]:

$$HO_2^{\cdot} + H_2O_2 \rightarrow HO\cdot + H_2O + O_2 \tag{2}$$

$$2HO_2^{\cdot} \rightarrow H_2O_2 + O_2 \tag{3}$$

$$HO_2^{\cdot} + HO\cdot \rightarrow H_2O + O_2 \tag{4}$$

According to Litter [24], excess of •OH can also cause its recombination to form H_2O_2 molecules.

B. UV-C/TiO₂ and UV-C/TiO₂/H₂O₂ MTX Degradation Experiments

Photocatalysis showed a similar behavior for UV-C and UV-C/H_2O_2 MTX degradation experiments (section III.A). The addition of H_2O_2 improved considerably MTX degradation and acidic pH presented the best results (Fig. 4).

In the presence of TiO₂, photocatalyst isoelectric point confers extra importance to initial pH. As TiO₂ isoelectric point is around 6.5 [14], at lower solution pH its surface would be positively charged, whereas at higher pH the surface would be negatively charged [28]. Isoelectric point of methotrexate, with pKa values of 4.7 and 5.5 [29], was calculated to be 5.1. Thus, at pH 3.5, TiO₂ as well as MTX must have a positive charge and repulsive forces should have caused low degradation. Therefore, at pH 7.0 MTX degradation is favoured when TiO₂ is near to neutrality and MTX only slightly negatively charged. Low degradation is also expected at pH 9.5, when both chemicals possess negatively charged surfaces. Nevertheless, as the best results were achieved at pH 3.5 for both processes (UV-C/TiO₂ and UV-C/TiO₂/H_2O_2), authors infer that the repulsive forces between particles do not play an essential role in these experimental conditions.

Another possible explanation is the low MTX solubility at low pH. MTX water solubility is very low [29], precipitating the compound in acidic urine, thus urine alkalization above pH 7.5 is required for patients [30], [31]. MTX crystal formation and deposition at pH 3.5 could explain the apparent higher molecule degradation under these conditions.

Somensi *et al.* [32] degraded MTX by ozonolysis and sonolysis/ozonolysis methods at pH ranging from 3.0 to 11.0, determining that both processes present the same efficiency at all tested pHs, so pH adjustment is not required when applying those processes. In the photocatalytic conditions studied here, a remarkable difference between degradation percentage was observed at the different pHs studied (Fig. 4).
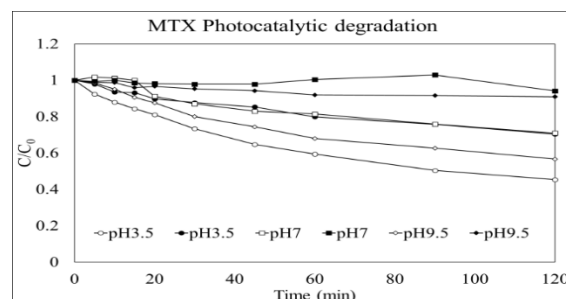

Fig. 4. MTX degradation in the UV-C/TiO₂ (black markers) and UV-C/TiO₂/H_2O_2 (white markers) processes at three different initial pHs: 3.5 (circles), 7.0 (squares) and 9.5 (rhombuses).

C. Kinetic Analysis

Table I presents the calculated rate constants and half-life times for UV-C/H_2O_2 and UV-C/TiO₂/H_2O_2 MTX degradation processes, which follow a first order reaction kinetic model for the first 30 min of reaction. As the best results were achieved with added H_2O_2, kinetic parameters for processes without added peroxide are not shown. Even though MTX degradation percentage is higher under acidic pH in UV-C/H_2O_2 processes (Fig. 3), higher rate constant and shorter τ were found at alkaline conditions (Table 1). When hydrogen peroxide is added to the UV_{254} process, better results are expected due to its photolytic dissociation, which yields hydroxyl radicals and provides an additional pathway to oxidation [33]. Comparing UV-C against UV-C/H_2O_2 and UV-C/TiO₂ against UV-C/TiO₂/H_2O_2, such improvement proves to be truth. For example, for UV-C/TiO₂ at pH 3.5, a k of 0.0045 min⁻¹ and a τ of 154 min were calculated, whereas the UV-C/TiO₂/H_2O_2 experiment at the same pH yielded a k of 0.0099 min⁻¹ and a τ of 70 min.

On the other hand, when comparing *k* values of UV-C

against UV-C/TiO$_2$ processes, with and without addition of H$_2$O$_2$, becomes evident that photolytic processes are better for MTX degradation under the three tested pHs, because photolytic *k* are more than twice the photocatalytic k values. A possible explanation for this effect is the direct reaction of H$_2$O$_2$ with the TiO$_2$ surface holes (thus competing with the contaminant), and formation of less oxidant species such as HO$_2$·. Said effect does not happen in UV-C processes as TiO$_2$ is not present.

TABLE I: RATE CONSTANTS (K) AND HALF-LIFE TIMES (τ) FOR MTX DEGRADATION WITH ADDED 3 MM H2O2, AT THREE PHS. A) UV-C/H2O2 AND B) UV-C/TIO2/H2O2

a) UV-C/H$_2$O$_2$				
pH$_{initial}$	k (min^{-1})	R^2	τ (min)	% Degradation at 120 min
3.5	0.0281	0.9078	24.67	82.76
7	0.0194	0.9778	35.73	66.25
9.5	0.0382	0.8711	18.14	68.42
b) UV-C/TiO$_2$/H$_2$O$_2$				
pH$_{initial}$	k (min^{-1})	R^2	τ (min)	% Degradation at 120 min
3.5	0.0099	0.9886	70.01	55.22
7	0.005	0.8822	138.63	29.38
9.5	0.0077	09852	90.02	43.89

D. Ecotoxicity Dynamics

Ecotoxicity measurement of aliquot samples taken at times 0, 60 and 120 min for each experiment with added H$_2$O$_2$ are shown in Table II. Initial sample of MTX photolytic degradation at pH 3.5 and 3 mM H$_2$O$_2$ caused a 20% diminution of *Vibrio fischeri* luminescence, meaning that MTX is a toxic substance. Ecotoxicity analysis of aliquot samples taken at 60 min, suggests that contaminant degradation generated byproducts with higher toxicity than the original molecule (Table II, Fig. 5). Ecotoxicity of samples taken at the end of each experiment shows that even if the toxicity decreases in the second half of the process, it is still higher than the original one, presumably by the combination of original contaminant and its byproducts. This ecotoxicity decrease in the second half of the experiment points to the possible need for extending the duration of experiments.

On the other hand, photocatalytic MTX degradation at pH 3.5 and added H$_2$O$_2$ increases sample toxicity, but not posterior decrease was observed (Table II). Then, it is concluded that MTX degradation by photolytic and photocatalytic treatments follows different pathways.

E. Residual H$_2$O$_2$

Remnant peroxide in photocatalysis experiments averaged 23.9 % whereas photolysis consumed around 57 % of applied oxidant. Lutterbeck *et al.* [6] suggest the addition of 9.8 mM H$_2$O$_2$, whereas results presented in this work suggest that peroxide dosage can be lowered up to around 2.5 mM.

F. MTX Byproducts Formation

Analysis by HPLC-MS spectrophotometry of samples from UV-C/H$_2$O$_2$ and UV-C/TiO$_2$/H$_2$O$_2$ MTX degradation processes at pH 3.5 showed the existence of four possible MTX degradation byproducts (Fig. 5).

Starting at methotrexate, a ketonization occurs at C(13), resulting in SP1 molecule with higher molecular weight. When the N bond in position 23 breaks, SP2 is formed. SP3 and SP4 molecules are formed if the bond from N in the position 14 breaks. These results match the suggested by Hsu *et al.* [34].

TABLE II: ECOTOXICITY VALUES FOR MTX DEGRADATION UNDER PHOTOLYTIC AND PHOTOCATALYTIC PROCESSES AT PH 3.5

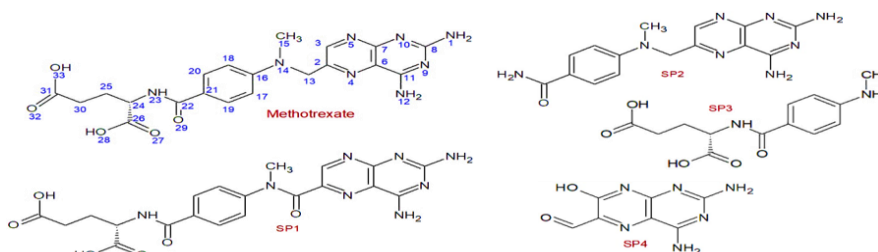| Process	Time (min)	EQUITOX	R^2
Photolysis	0	19.334	0.9140
	60	147.883	0.9978
	120	37.383	0.9943
Photocatalysis	0	16.485	0.9729
	60	21.504	0.9091
	120	27.104	0.9821

Fig. 5. Possible TMX degradation byproducts.

IV. CONCLUSIONS

The degradation of MTX under photolysis and heterogeneous photocatalysis (UV and UV/TiO$_2$) processes, despite being incomplete (up to 82% and 57% degradation respectively), was highly enhanced by the addition of 3 mM H$_2$O$_2$ and by acidic pH. The proposed byproducts of MTX degradation still have a toxic impact on the environment.

A feasible way to obtain better results would be connecting several reactors in series or increasing experimental time, these solutions could achieve total removal of the initial MTX and a higher mineralization.

Tested reactors represent an option for the removal of emerging contaminants, especially drugs, if a systematic sensitivity analysis of essential variables of the corresponding processes is carried out.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

García-Roig and Proal-Nájera conducted the research and facilitated the resources; González-Burciaga and Núñez-Núñez wrote the paper; González-Burciaga and García-Prieto performed the experiments and analyzed the data. All authors have approved the final version of this article.

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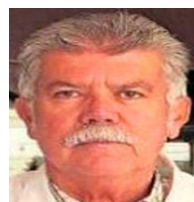
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