

Article

Cytostatic Drug 6-Mercaptopurine Degradation on Pilot Scale Reactors by Advanced Oxidation Processes: UV-C/H₂O₂ and UV-C/TiO₂/H₂O₂ Kinetics

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Abstract: 6-Mercaptopurine (6-MP) is a commonly used cytostatic agent, which represents a particular hazard for the environment because of its low biodegradability. In order to degrade 6-MP, four processes were applied: Photolysis (UV-C), photocatalysis (UV-C/TiO₂), and their combination with H₂O₂, by adding 3 mM H₂O₂/L (UV-C/H₂O₂ and UV-C/TiO₂/H₂O₂ processes). Each process was performed with variable initial pH (3.5, 7.0, and 9.5). Pilot scale reactors were used, using UV-C lamps as radiation source. Kinetic calculations for the first 20 min of reaction show that H₂O₂ addition is of great importance: in UV-C experiments, highest k was reached under pH 3.5, k = 0.0094 min^{−1}, while under UV-C/H₂O₂, k = 0.1071 min^{−1} was reached under the same initial pH; similar behavior was observed for photocatalysis, as k values of 0.0335 and 0.1387 min^{−1} were calculated for UV-C/TiO₂ and UV-C/TiO₂/H₂O₂ processes, respectively, also under acidic conditions. Degradation percentages here reported for UV-C/H₂O₂ and UV-C/TiO₂/H₂O₂ processes are above 90% for all tested pH values. Ecotoxicity analysis of samples taken at 60 min in the photolysis and photocatalysis processes, suggests that contaminant degradation by-products present higher toxicity than the original compound.

Keywords: 6-mercaptopurine photodegradation; wastewater; cytostatic; emerging pollutants; photocatalysis

1. Introduction

Emerging pollutants (EPs) could be defined as a new class of products, synthetic or natural, that have found their way into humans and animals due to their recent discovered sources [1–3]. Nowadays, there are no guidelines or legislative background for EPs management; for that reason, these pollutants are not commonly monitored in the environment or included in routine monitoring programs and their fate, occurrence, and environmental effects are not well understood [1,2]. Drugs are a major group of EPs that have been detected in wastewaters, surface waters, groundwater and drinking water. In the last decade their presence has been reported in the aquatic cycle in the order of ng/L and in some cases a few µg/L, having a negative impact on the environment. [4–7].

Cytostatic drugs, also called anticancer or antineoplastic agents, are used in hospitals for the treatment of cancer via chemotherapy. These drugs represent a high risk regarding human health and a particular hazard for the environment because of an increasing demand for the chemotherapy treatment and their carcinogenic, genotoxic, mutagenic, and cytotoxic properties, even at low concentrations, added to a low biodegradability. Therefore, it is crucial to monitor their presence in the environment to extend the research

in order to classify their by-products, as they have biotoxic potential [4–6,8,9]. Different organisms have been used to assess the toxicity of certain compounds in the environment, including aquatic bacteria *Vibrio fischeri* [6,10,11], which emits light under normal conditions. When the enzymatic activity of this organism is affected by toxins, bioluminescence decreases [12]. Advances in analytical instruments have allowed the detection of cytostatic drugs and the further transformation by-products in environmental samples, even at very low concentration (ng/L). Recognizing the presence of these compounds has driven the research on potential methods for their removal from water bodies [4,5,7].

Many studies have been carried out with different processes for the removal of cytostatic drugs. Zhang et al. 2013 [13], conducted a review article on the removal of cytostatic drugs present in aquatic environments; in the cited works, 21 compounds were found in hospital effluents, influents and effluents from wastewater treatment plants, and surface waters by different detection methods like SPE GC-MS, LC-MS/MS, SPE HPLC-MS/MS, LLE GC-MS, SPE UPLC-MS/MS, ICP-MS, among others. The authors gathered information from studies between the years 2005–2012, where cytostatic drugs were removed or degraded by advanced technologies, such as membrane bioreactors, membrane filtration methods, electrochemical oxidation, catalytic oxidation, UV/H₂O₂, and ozonation, with results ranging from 20% to 100%.

In 2017, a research carried out by Franquet-Griell et al. [14] explored the degradation of 16 cytostatics commonly detected in hospital effluents, through hydrolysis, biodegradation in a sequential batch reactor, UV-C process, UV-H₂O₂, and simulated sunlight reactor. The best results for all pollutants were achieved with the UV/H₂O₂ process.

Antineoplastic drugs play an essential role in the development of chemotherapy treatment [15]. 6-mercaptopurine (6-MP) is an anticancer agent that has the additional application as immuno-suppressive and anti-inflammatory agent [15,16]. 6-MP has been widely used for the treatment of acute lymphoblastic leukemia, chronic myeloid leukemia, choriocarcinoma, Crohn's disease, and psoriatic arthritis [15–18]. When it is administered in chemotherapy, other drugs must be used to extend the duration of remission achieved [19], which can last from two to three years after diagnosis [20]. The initial dose in Mexico for adults varies from 75 mg/m²/day [21] to 100 mg/m²/day [22–24]. Whereas the starting dose in the United Kingdom, the Nordic countries and the United States is 75 mg/m²/day, in most of Europe, the initial dose is 50 mg/m²/day [20]. The percentage of drug excreted unchanged through the urine is 50%, so half of the high doses of 6-MP administered for cancer treatment end up in sewage [13]. The doses of 6-MP for the treatment of diseases such as intestinal arterial insufficiency, Crohn's disease, ulcerative colitis and inflammatory bowel disease, range from 1 to 2.5 mg/kg/day [25,26]. Due to their characteristics, high doses and administration over large periods of time, presence and removal of cytostatic drugs as 6-MP from wastewater, have become a challenge.

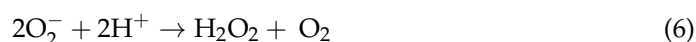
Heterogenous photocatalysis (HPC) has become the most distinctive, popular, effective and promising treatment technique for the removal of recalcitrant contaminants, such as pharmaceutical wastewater. The mechanism of HPC consists in the capability of the semiconductors to generate hydroxyl radicals (•OH) in situ, which are extremely reactive and strong oxidizing agents ($E^\circ = 2.8$ eV), and can lead to further reactions generating harmless products as CO₂ and H₂O [27–30]. The photocatalyst is the core of the photocatalytic process, most photocatalysts are metal oxides such as: titanium dioxide (TiO₂), zinc oxide (ZnO), zinc sulfide (ZnS), tungsten trioxide (WO₃), cadmium sulfide (CdS), tin dioxide (SnO₂), and gallium phosphide (GaP), among others [31,32]. TiO₂ is widely used in environmental applications due to its low cost and properties, such as chemical stability, light absorption, it is biologically inert and resistant to chemical corrosion, and can be used at ambient temperature and pressure [27–29,32].

HPC involves a series of simultaneous oxidative and reductive reactions on the surface of the semiconductor, initiated by the irradiation of TiO₂ with UV light to excite the catalyst (Equation (1)). When the energy becomes equal to or greater than the band gap, the electrons in the valance band (e^-_{VB}) are promoted to the conduction band (CB) leaving

behind a hole (h^+_{VB}) in the surface of the photocatalyst. While the h^+_{VB} is positive enough to generate $\bullet OH$ in the surface of the photocatalyst that contains absorbed water (Equations (2) and (3)), the CB with a negative charge, reduces the oxygen in the solution in order to produce another series of $\bullet OH$ (Equations (4) and (5)) [27–30]:



$\bullet OH$ could also be generated through reactions between some intermediate products:



Formed $\bullet OH$ reacts with organic compounds R (either chemical compounds or microorganisms) present in the sample, generating a mineralization reaction:



The objective of this work was to apply photolytic and photocatalytic processes on pilot scale reactors, using UV-C lamps, for the degradation of 6-MP at different conditions (acidic, basic and neutral media), and comparing the results when hydrogen peroxide (H_2O_2) is added as an oxidant in the processes. It also analyses the toxicity of 6-MP and possible transformation by-products, generated during experimentation, using marine bioluminescent bacteria *V. fischeri*.

2. Results and Discussion

2.1. UV-C and UV-C/ H_2O_2 6-MP Degradation Experiments

Experiments performed in the absence in radiation (control experiments), showed a degradation of 20% after 80 min of reaction, so the effect of H_2O_2 addition alone is not enough to reach the degradation data here presented for experiments with radiation.

The 6-MP degradation reached through 2 h UV irradiation only, was lower than 70% at the three tested pH values. Direct photolysis of molecules present in wastewater is possible when irradiating at 254 nm wavelength, but the process is not effective to achieve total mineralization of contaminants [33]. According to Smaranda et al. [34], an energy of 3.13 eV can excite electrons present in 6-MP; given this, radiation under 396 nm wavelengths will excite the molecule. As radiation used in this research was 254 nm, breaking of the molecule was expected. In the past, UV light was reported to be responsible for photodegradation of azathioprine, a parent compound to 6-MP [34]. In fact, 6-MP presents maximum absorbance at approximately 340 nm [35] and in aqueous solution, generates reactive oxygen species (ROS) when irradiated with UV radiation in the presence of molecular oxygen [35,36]. Thiopurines are known to absorb UV-A radiation and are converted to an unstable excited triplet state that can interact with oxygen to form a thiopurine radical and superoxide [35]. Superoxide radical ($O_2^{\bullet -}$) is able to degrade substituted aromatic compounds with high absorption in the UV range but has a low oxidizing power [37].

Figure 1 shows 6-MP degradation by UV-C and UV-C/ H_2O_2 experiments. The best results (6-MP degradation > 99%), were achieved at initial pH of 3.5 and 7, when H_2O_2 was added.

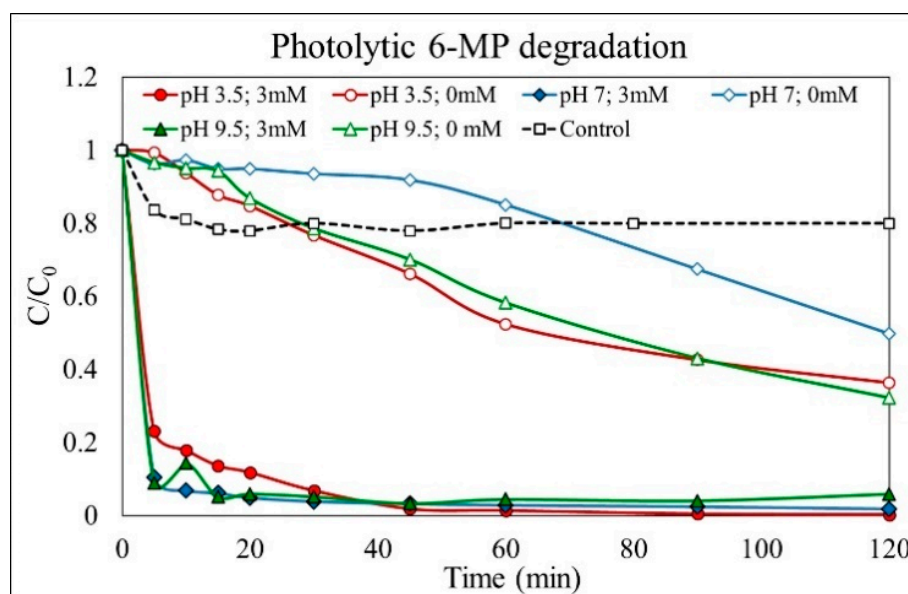
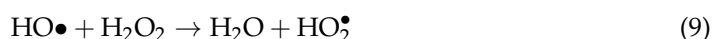
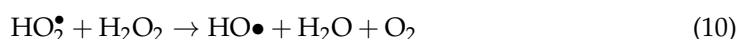


Figure 1. 6-MP degradation in the UV-C (empty markers) and UV-C/H₂O₂ (filled markers) processes with and without H₂O₂ addition and three different initial pH: 3.5 (circles), 7 (rhombuses) and 9.5 (triangles). Control experiment is represented by the segmented line.

Irradiation below 280 nm wavelength, breaks O-O bonds of H₂O₂ into HO• [38], thus degradation enhancement is to be expected. Best degradation percentage after only 5 min treatment is in fact achieved in alkaline conditions; however, the best results at the end of treatment (120 min) were achieved at acidic and neutral pH, being a possible explanation the formation of less reactive HO₂• radicals in HO• excess, through (Equation (9)), this is similar to what other authors observed [34,38–40]:



HO₂• radicals can later react as follows [40]:



2.2. UV-C/TiO₂ and UV-C/TiO₂/H₂O₂ 6-MP Degradation Experiments

Experiments performed in the absence in radiation (control experiments), showed a degradation of 16% after 80 min of reaction, so the effect of H₂O₂ addition alone is not enough to reach the degradation reached by the experiments in the presence of radiation.

Photocatalysis showed a similar behavior than UV-C and UV-C/H₂O₂ 6-MP degradation experiments (Section 2.1): addition of H₂O₂ improved considerably 6-MP degradation. When no H₂O₂ is added, a fast concentration drop is presented under acidic condition, but after 120 min of treatment, alkaline conditions present better results (Figure 2).

In the presence of TiO₂, photocatalyst isoelectric point confers extra importance to initial pH. As TiO₂ isoelectric point is around 6.5 [41], at lower solution pH its surface would be positively charged, whereas at higher pH the surface would be negatively charged [42]. The isoelectric point of 6-MP is 9.47, calculated from its pK_a values of 7.77 and 11.17 [43]. Nevertheless, the performance of UV-C/TiO₂/H₂O₂ is more efficient that obtained with UV-C/TiO₂ and no remarkable differences were observed at all tested pH when H₂O₂ was added (92.48–100% degradation), authors infer that the repulsive forces between particles

do not play an essential role in these experimental conditions and the importance of adding H_2O_2 is evident.

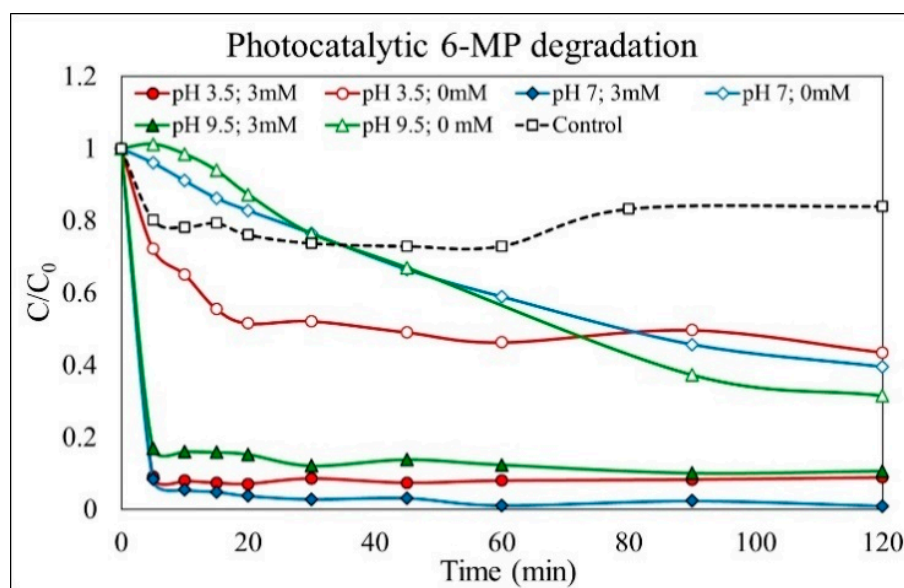


Figure 2. 6-MP degradation in the UV-C/ TiO_2 (empty markers) and UV-C/ TiO_2 / H_2O_2 (filled markers) processes with and without H_2O_2 addition and three different initial pH: 3.5 (circles), 7 (rhombuses) and 9.5 (triangles). Control experiment is represented by the segmented line.

2.3. Kinetic Analysis

Table 1 presents calculated photolytic constants (k_{ph}), operational photocatalytic constants (k_{op}) and half-life times ($t_{1/2}$) for the tested 6-MP degradation processes, which follow a first order reaction model for the first 20 min of reaction. In processes where no H_2O_2 was added (UV-C and UV-C/ TiO_2), higher constants and shorter half-life times are reached under acidic conditions (Table 1). It is known that NO_3^- and NO_2^- present in water, when excited under light, result in the formation of $\text{HO}\bullet$ [33]; as HNO_3 was used to lower the initial pH of the solution, nitric acid dissociation is to be blamed by the presence of such ions, which provides an additional pathway for radical formation, explaining the better results found under a 3.5 pH.

Even though 6-MP maximum degradation (100%) achieved with UV-C/ H_2O_2 processes is higher under acidic and neutral pH (Table 1), higher k_{ph} and shorter $t_{1/2}$ were found at alkaline conditions. Best results should appear at alkaline conditions experiments given that the conjugate anion of H_2O_2 increases with pH and favors $\text{HO}\bullet$ production [37].

When H_2O_2 is added to the photolysis UV₂₅₄ process, better results are expected due to its photolytic dissociation, which yields hydroxyl radicals and provides an additional pathway to oxidation [44]. Analyzing the processes where H_2O_2 was added against the ones without it, such improvement proves to be truth.

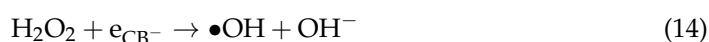
When comparing K_{ph} values of UV-C/ H_2O_2 against K_{op} values of UV-C/ TiO_2 / H_2O_2 processes, it becomes evident that photocatalytic processes are better for 6-MP degradation, because photocatalytic constant values are higher than the photolytic, except in the case of UV-C/ H_2O_2 process under an initial pH of 9.5, which yielded the highest K. On the other hand, when comparing the K_{ph} of the processes with H_2O_2 against the K_{ph} of the processes without it, the importance of adding the oxidant to the reaction becomes clear. The same behavior can be observed in the photocatalysis processes.

Table 1. Rate photolytic constant (k_{ph}), operational photocatalytic constants (k_{op}) and half-life time ($t_{1/2}$) for 6-MP degradation with and without H_2O_2 added, at three pH.

Process	pH _{initial}	k_{ph} (min ^{−1})	R ²	$t_{1/2}$ (min)	Degradation % at 120 min
UV-C	3.5	0.0094	0.9562	73.74	65.60
	7	0.0025	0.7494	277.26	52.24
	9.5	0.0063	0.8622	110.02	69.90
UV-C/ H_2O_2	3.5	0.1071	0.8076	6.47	100
	7	0.1616	0.7693	4.29	100
	9.5	0.1975	0.7174	3.51	98.34
Process	pH _{initial}	k_{op} (min ^{−1})	R ²	$t_{1/2}$ (min)	Degradation % at 120 min
UV-C/ TiO_2	3.5	0.0335	0.9340	20.69	58.60
	7	0.0099	0.9970	70.01	61.70
	9.5	0.0072	0.8198	96.27	71
UV-C/ TiO_2 / H_2O_2	3.5	0.1387	0.6183	4.99	94.40
	7	0.1725	0.7614	4.02	100
	9.5	0.0857	0.5526	8.09	92.48

As K values were calculated using 20 min of reaction data, R² values are greatly affected by the fast degradation in the first 5 min of the processes where H_2O_2 is added. As can be seen in Figures 1 and 2, a rapid descent in concentration was measured in the first 5 min of reaction, so the line drawn between first and last experimental points (0 and 20 min for kinetic calculations) leaves out the points in between, resulting in low R² values.

When H_2O_2 is added, hydroxyl radicals can be generated through decomposition from hydroxide peroxide when irradiated (Equation (13)), but also by the contact of hydrogen peroxide and the electron in the conduction band of the titanium dioxide (Equation (14)), reinforcing pollutant degradation by increasing OH• production [33,34]:



2.4. Ecotoxicity Dynamics

According to Cramer's rules, 6-MP has a toxicity level III (Toxtree database version 3.1.0), which means that initial samples of 6-MP caused more than 20% diminution of *Vibrio fischeri* bioluminescence.

Ecotoxicity analysis of samples taken at 60 min in the photolysis and photocatalysis processes, suggests that contaminant degradation generated by-products with higher toxicity than the original compound. 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. A similar behavior was observed in the past by Chatzimpaloglou et al. 2021 [45], for photocatalytic degradation of the antineoplastic drug irinotecan, and concluded that the toxicity increase is due to transformation products formation at the first minutes of the process.

The increase in toxicity at 60 min of experimentation is due to two by-products, purine-6-sulfinate and purine-6-sulfonate. According to Moore (1998), these compounds are the main oxidation products of 6-MP and generate strongly oxidizing SO_2^- and SO_3^- radicals with extensive reactivity, capable of breaking DNA. The presence of these compounds, in addition to traces of 6-MP, increases the toxicity towards the end of the process. The

toxicity decreases with the subsequent degradation of the remnant of the parent compound and the by-products, but as mineralization is not reached, the solution remains toxic [46].

2.5. Residual H_2O_2

Remnant peroxide in photocatalysis experiments averaged 36.94% whereas photolysis consumed around 67.02% of applied oxidant. The results presented in Figure 3 suggest that H_2O_2 dosage can be lowered up to around 2 mM for experiments of 120 min, and the rapid decrease in concentration in the first five minutes of experimentation seems to demonstrate that a rapid formation of hydroxyl radicals under 254 nm radiation is the main source of degradation. Rapid degradation of H_2O_2 in the first 5 min of reaction, matches fast degradation of pollutant measured in the same time period.

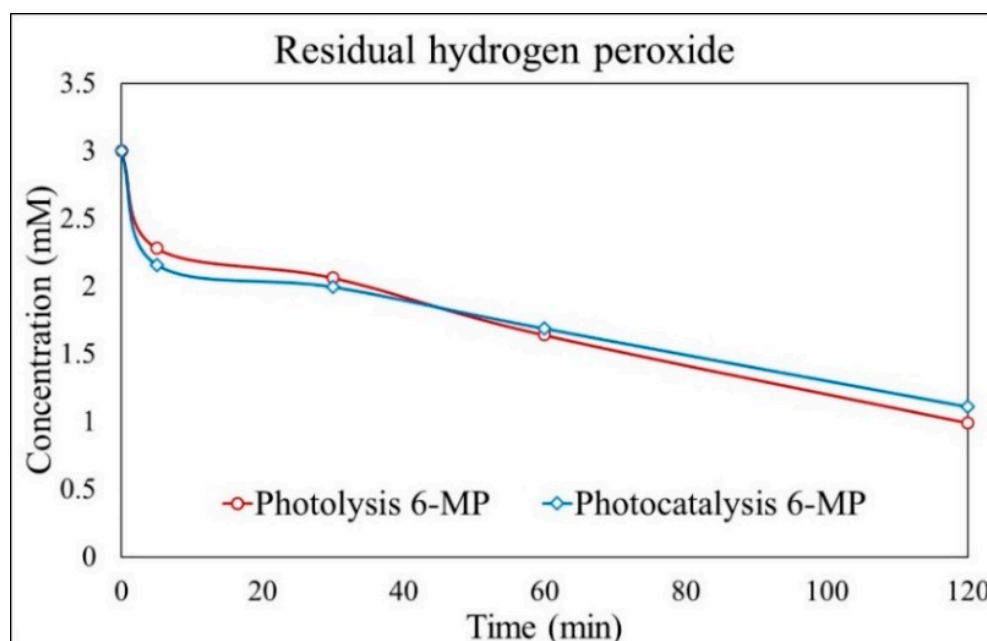


Figure 3. Hydrogen peroxide consumption during 6-MP photolysis (circles) and photocatalysis (rhombuses) degradation.

2.6. 6-MP by-Products Formation

Analysis by HPLC-MS of samples from UV-C/ H_2O_2 and UV-C/ TiO_2 / H_2O_2 6-MP degradation processes were performed in normal phase and reverse phase, and showed the existence of various degradation by-products. Starting from 6-mercaptopurine in the presence of dissolved oxygen and favored in basic pH, anionic salts purine-6-sulfinate and purine-6-sulfonate were found in the analysis of the samples taken. Other possible product can be hypoxanthine, caused by the decrement of oxygen in the solution. These results match the suggested by Hemmens and Moore [36]. The increase of free radicals produced by the photocatalytic processes and the acidic pH of the solution, favors the creation of 7H-purine-6-sulfonic acid, also found in the HPLC-MS analysis. The photodegradation pathway may occur as shown in the Figure 4.

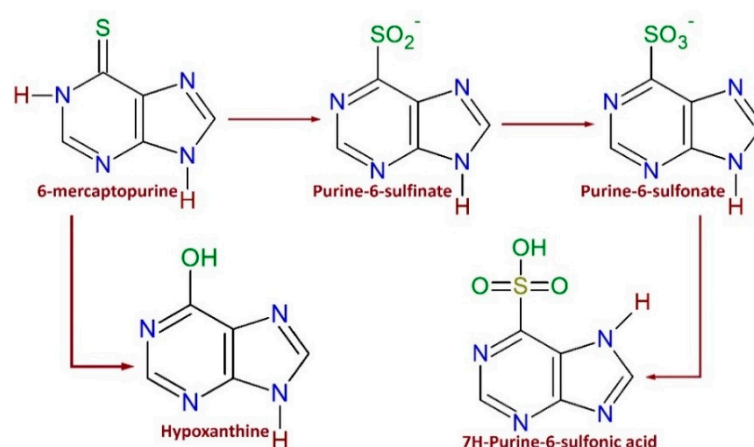


Figure 4. Possible 6-MP degradation pathways.

3. Materials and Methods

3.1. Sample Preparation and Reagents

Samples were produced by dissolving the commercial drug 6-MP (Purinethol®, Aspen Pharmacare, La Lucia, Durban, South Africa) in distilled water to an initial concentration of 6 mg/L, based on the works of degradation of cytostatics by heterogeneous photocatalysis carried out by Molinari et al. 2008 [47], and Evgenidou et al. [48], where the initial concentrations of contaminant were 8 mg/L and 1 mg/L, respectively. Due to reactors characteristics, in UV-C experiments, a sample volume of 50 L was used, meanwhile UV-C/TiO₂ required volume was 25 L. H₂O₂ was obtained from Labbox Labware (CAS: 7722-84-1, Barcelona, Cataluña, Spain). NaCl used for the ecotoxicity method was purchased from Panreac Química (CAS: 7647-14-5, Barcelona, Cataluña, Spain) and the bacteria *Vibrio fischeri* was part of the commercial kit WATERTOX™ (Environmental Bio-detection Products Inc., Burlington, ON, Canada). Titanium oxysulfate was purchased from Sigma-Aldrich (CAS: 13825-74-6, St. Louis, MO, USA).

3.2. UV-C and UV-C/H₂O₂ 6-MP Degradation Processes

UV reactor used for UV-C and UV-C/H₂O₂ experiments is shown in Figure 5, modified from Núñez-Núñez et al. [41]. The reactor system consists of a 200 L tank for the water sample, a 1 hp pump for sample recirculation through the system, a 50 µm filter to prevent large particles from entering the main reactor body, and a rotameter in order to measure the water flow going into the reactor. The reactor, main part of the system, is a compartment of stainless steel with the inlet on the bottom part and the outlet on the top.

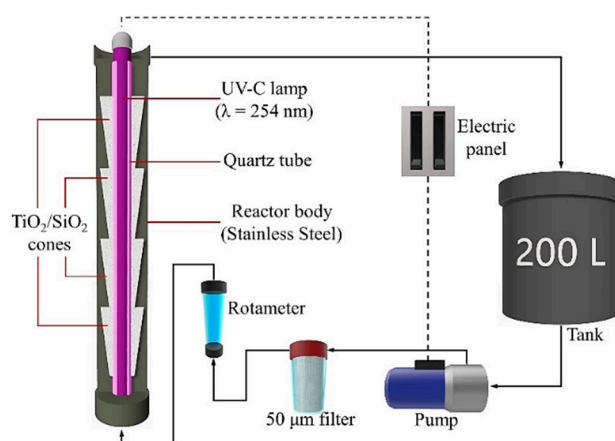


Figure 5. Reactor system used for UV-C and UV-C/H₂O₂ 6-MP degradation processes.

A low-pressure mercury lamp (254 nm radiation peak; T5 Philips, Amsterdam, The Netherlands) was used as irradiation source. Such lamp was placed inside of a transparent quartz tube to prevent it from entering in contact with the sample. The tube is introduced on 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 [49,50].

UV-C and UV-C/H₂O₂ experiments were performed at 650 L/h flow rate. Once sample recirculation started in the system, pH was adjusted with 65% (v/v) HNO₃ or 0.1 M NaOH solutions, to tested pH 3.5, 7 and 9.5. pH values were selected given the TiO₂ isoelectric point of 6.5: an acidic pH, were TiO₂ surface is positively charged; basic pH, were TiO₂ surface is negatively charged; and neutral pH, were TiO₂ superficial charge is low.

The effect of H₂O₂ on the degradation of 6-MP was also tested, the experiments were carried out with the addition of 3 mM H₂O₂/L and the absence of it at each pH. After pH adjustment and H₂O₂ 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 6-MP degradation.

3.3. UV-C/TiO₂ and UV-C/TiO₂/H₂O₂ 6-MP Degradation Processes

For photocatalysis degradation processes, the commercial AOP1 model reactor (Bright-Water Environmental, Harleston, Norfolk, UK) was used. This reactor is composed by a titanium cylinder covered in the internal wall by a TiO₂ layer. Radiation is provided by a lamp emitting radiation at 254 nm and sample recirculation is achieved by a 0.85 kW water pump (Jet Inox 850, super-ego tools, Abadiano, Vizcaya, Spain). Figure 6 shows the reactor, which is 75 mm in 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/H₂O₂ experiments: same tested pH values (3.5, 7, and 9.5), and in the presence or absence of H₂O₂ (0 and 3 mM).

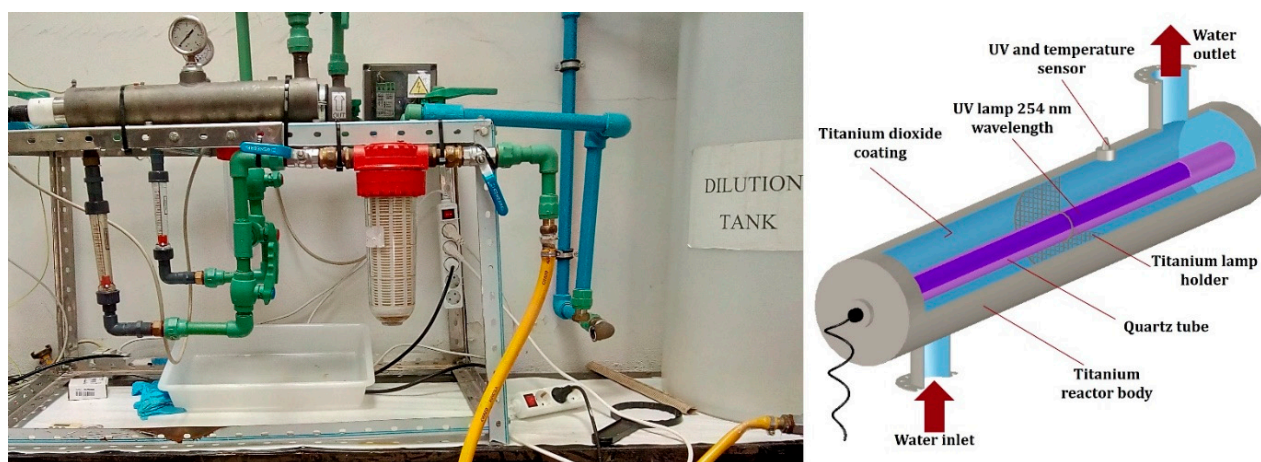


Figure 6. Commercial photocatalytic UV reactor used in UV-C/TiO₂ and UV-C/TiO₂/H₂O₂ 6-MP degradation processes (left); Titanium AOP1 reactor (right).

3.4. Control Experiments

In order to measure the effect of hydrogen peroxide on samples, control experiments in absence of radiation were performed. Sample was prepared as described above and 3 mM of H₂O₂/L of sample was added. The recirculation was started, but the lamp was kept off. Degradation was measured at different times. Control experiments were performed for both photocatalysis and photolysis processes, under an initial sample pH of 7.

3.5. Chemical Analysis

3.5.1. 6-MP Analysis

Water samples at initial time (0 min) were taken directly from the solution tank; subsequent samples were taken at the end of the system, right where it connects to the solution tank. All samples were analyzed by UV–VIS spectrophotometry (T80+ UV–VIS spectrophotometer, PG Instruments Ltd., Alma Park, Leicestershire, UK) at 324 nm wavelength to measure 6-MP concentration. Calibration curve ($R^2 = 0.9996$) was built using 6-MP monohydrated purchased from Sigma-Aldrich (CAS: 6112-76-1, St. Louis, MO, USA), dissolved in deionized water.

3.5.2. Ecotoxicity Measurement

Water samples taken at 0, 60, and 120 min reaction times from selected experiments were tested for ecotoxicity in order to measure and compare potential environmental damage by 6-MP and its photodegradation by-products. Such analyses were performed by the UNE-EN ISO 11348-3 method [51], which measures the effect of the sample on *V. fischeri* luminescence. Luminescence was measured with a luminometer Bacterial Systems BG-1 (GEM Biomedical Inc., Lewisville, TX, USA). Experiments selected for the analysis were photolysis, under initial pH of 3.5 and photocatalysis, under initial pH of 7, both with H_2O_2 addition.

3.5.3. Residual H_2O_2 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 Klammerth [52], which is based in the formation of a yellow solution when H_2O_2 and titanium oxysulfate are mixed, absorbance of the solution is then measured at 410 nm wavelength. Calibration line was built using H_2O_2 solutions with concentrations ranging from 0.5 up to 3 mM.

3.5.4. 6-MP by-Products Formation

Water samples from experiments were analyzed by mass spectrometry in order to determine the possible chemical structures of 6-MP degradation by-products. 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 HPLC Surveyor MS with a LTQ spectrometer.

3.6. Kinetic Analysis

Analysis of 6-MP concentration at different times of the reaction were used to determine reaction operational photocatalytic constants (k_{op}) and photolytic constants (k_{ph}) of the first order reaction by plotting $\ln[6\text{-MP}]$ against time in the first 20 min of reaction [41,53]. Once k_{op} and k_{ph} were determined, half-life time ($t_{1/2} = \ln 2/k_{op}$; $t_{1/2} = \ln 2/k_{ph}$) of 6-MP degradation of each process was calculated.

4. Conclusions

Experiments show that 6-MP is degraded by both UV-C and UV-C/ TiO_2 processes, and degradation higher than 50% can be achieved, under acidic, neutral, or basic pH conditions. Hydrogen peroxide addition to photolysis and photocatalysis experiments greatly improves 6-MP degradation, as degradation percentages here reported for UV-C/ H_2O_2 and UV-C/ TiO_2 / H_2O_2 processes are above 90% for different initial pH values.

pH lowering through HNO_3 addition, improves degradation as kinetic results signal, but such effect can be observed only in absence of H_2O_2 ; when the oxidant is present, its effect is the most important factor in the experiments, which points to $HO\bullet$ formation by H_2O_2 molecule rupture under UV-C radiation provided. Nevertheless, special care should be put into the hydrogen peroxide addition, as an excess could result in a detrimental effect

over degradation. In this research, H₂O₂ addition (3 mM/L) ended up being excessive, so a dose of 2 mM, instead of 3, should be tested for 2 h duration experiments.

Even though, when H₂O₂ is added to the processes, 6-MP degradation over 80% is reached in the first 10 min of reaction, toxicity actually increased, demonstrating that byproducts are formed, and such byproducts, or their combination with remaining 6-MP, possess a greater toxicity than the original compound.

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