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Heterogeneous photocatalytic degradation, mineralization and detoxification of ampicillin under varying pH and incident photon flux conditions

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ABSTRACT

Most antibiotics have been demonstrated to be low biodegradable in biological treatment systems, thus advanced oxidation processes, particularly solar photocatalytic oxidation also known as a green process, have gained an essential attraction for their effective removal from effluents. However, so far there has been a very limited number of studies on ampicillin (AMP) removal that focused on one initial concentration of AMP and fixed photon energy. Therefore, this study was designed to investigate the effect of varying pH and incident photon fluxes on heterogeneous photocatalytic AMP removal at two AMP initial concentrations (50 and 100 mg L^{-1}) using 0.5 g L^{-1} TiO_2 dose under UV-A (315–400 nm wavelengths) irradiation. Photocatalytic experiments were run in a vessel with 200-mL effective sample volume. Process efficiency was monitored by degradation (UV–vis, LC-MS/MS), mineralization (TOC), and acute toxicity to *Daphnia magna* during 24–48 h exposure times to evaluate possible toxic effect of oxidation by-products. Toxicity results and TOC removal (30–60 min) indicated simultaneous mineralization and degradation of both initial AMP concentrations. Lower ratio of TiO_2/AMP, as one of the key factors affecting the oxidation efficiency, promoted increasing formation of by-products that interacted with surface chemistry on TiO_2 nanoparticles leading to a process efficiency decrease at 100 mg L^{-1} AMP initial concentration. When photon energy was increased, it enhanced the removal of AMP. A preliminary cost evaluation showed that a 3.62 mW cm^{-2} energy flux was satisfying to obtain over 50% of TOC removal and a nearly complete detoxification of 50 mg L^{-1} AMP.

Keywords: Ampicillin; Degradation; Detoxification; Photon energy; Minerilization; Heterogeneous photocatalysis; TiO_2; *Daphnia magna*

1. Introduction

Due to inefficient removal rates of pharmaceuticals in conventional biological processes, urban wastewater treatment plants (UWWTP) become potential promoters of surface water contamination and may pose a threat for the environment [1–5]. Antibiotics with their high consumption rates can pose severe hazards and risks to the aquatic environment [6–8]. Their continuous release into the receiving environment leads to

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increase in synergistic effects with other pharmaceuticals and chemicals or specifically, development of antibiotics resistance [9–12].

Integration of advanced oxidation processes (AOPs) to UWWTPs as a tertiary treatment phase can significantly contribute to removal of priority pollutants [13–15]. Based on hydroxyl radical formation, photocatalysis leads to non-specific reactions between hydroxyl radicals and organics in the reactor. Solar-driven photocatalysis, with a possible modification on the photocatalyst, was reported to be a green and clean detoxification technology [16,17]. During oxidation, mineralization proceeds as a slow process producing less stable by-products compared to the target compounds of concern. Thereby by-products may be more readily biodegradable than the parent compounds or may pose risks on the aquatic organisms [4,6,14,15].

Ampicillin (AMP) is used in human and veterinary medicine, and it is associated to cause antibiotic resistance to *E. coli* bacteria [18]. Among eastern European countries, Turkey was reported to be at the first order for antibiotics consumption [19]. In our previous work, regarding evaluation of predicted environmental antibiotic concentrations in sewage of Tekirdag city, Turkey, AMP was associated with the risk concern to aquatic environment [20]. The aqueous solutions of B-lactams, specifically AMP, have been subjected to various AOPs processes [21–23]. Elmolla et al. [23] introduced 105 mg L^{-1} AMP solution to photocatalytic reactor at pH 5 during 300 min of UVA (365 nm) illumination supplied with a nominal power of 6-W lamp positioned on the top of the reactor. Degradation levels of AMP were reported to be 33.3, 52.4, 54.3 and 52.4% at TiO_2 concentrations of 0.5, 1.0, 1.5 and 2.0 g L^{-1}, respectively. However, neither photon energy optimization nor ecotoxicity that are both important to optimize a photocatalytic reactor were considered in their study. Thus, this study aimed to investigate TiO_2-based heterogeneous photocatalytic process for removal of two initial AMP concentrations applying different levels of UV-A energy and varying pH values. The removal of AMP was monitored by UV–vis, TOC and LC-MS/MS while acute toxicity of by-products formed during photocatalytic oxidation was tested using *Daphnia magna* standardized test. A preliminary cost assessment was also given.

2. Materials and methods

2.1. Chemicals

AMP (CAS 56-75-3) was purchased from Sigma–Aldrich. AMP (molecular weight of 349.4 g mol^{-1}, and pK_a value of 7.3) is a semi-synthetic penicillin obtaining its antimicrobial properties from the presence of a β-lactam ring with a cyclic amide on which nitrogen atom is attached to the β-carbon. It has two ionizable groups, the amino group is in alpha position to the amide carbonyl group and the carboxyl group. AMP, similar to the most of the penicillin group antibiotics, absorbs light below 300 nm wavelength. The hydrolysis reaction would proceed through the attack of the nucleophile H_2O to the β-lactam ring followed by an opening ring as seen in Fig. 1 [6,22,23]. pK_a values and related surface functional groups are also shown in Fig. 1.

Titanium dioxide (TiO_2) was purchased from Sigma Aldrich (CAS Number 13463-67-7) with a specific surface area varying between 35 and 65 m^2/g (BET) and a mean particle size of 21 nm.

2.2. Solutions

Aqueous solutions containing 50 and 100 mg L^{-1} AMP were prepared by adding proper amounts of antibiotics in distilled water and slowly mixed for 30–60 min at room temperature in the closed flasks covered by aluminium paper. pH of the solutions was adjusted to the required value by 1 N H_2SO_4 or 1 N NaOH. Antibiotic solutions were prepared daily and stored at +4˚C during experiments.

2.3. Experimental

The experiments were performed in a batch reactor system (200-mL water volume) using a photoreactor

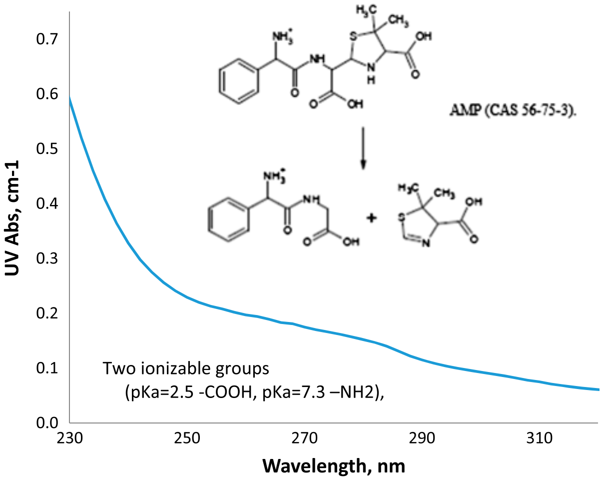

Fig. 1. Chemical structure and UV Spectrum of VAN-B also the degradation of ampicillin under acidic, basic and thermal degradation treatments [28].

equipped with multi-lamps (4 × 2 lamps were positioned at left and right sides +6 lamps were positioned on the top. Each UV-A lamp yields 8 W) as shown in Fig. 2. A series of incident photon fluxes (2.16, 2.69, 3.62 and 4.92 mW cm^{-2}) was attempted during experiments.

Before starting the photocatalysis process, a suspension of TiO_2 catalyst was prepared in a 5 mL of distilled water and it was exposed to ultrasonication for 5 min. After that, AMP solutions of 50 and 100 mg L^{-1} were transferred into the TiO_2-suspended vessels and placed into photoreactor. UV-A lamps were kept switched off during the dark adsorption vs. time during 120 min in order to observe adsorption potential of AMP on catalyst surfaces. A 30-min dark adsorption period was fixed during further experiments. Experiments were run vs. time (0–120 min) under continuous magnetic stirring conditions using TiO_2 catalyst dose of 0.5 g L^{-1} considering experimental conditions by Elmolla et al. [23]. Photocatalysis experiments were carried below the pK_a value of AMP at pH 5 as it was also reported previously elsewhere [24]. Effluent was filtered using Millex cut-off filter (0.45 μm) to remove residual TiO_2 before analysis. Photolysis experiments were also carried out under the same conditions with photocatalysis experiments as the control test.

All experiments were run at constant room temperature (25 ± 0.5˚C).

2.4. Analytic methods

The incident of photon flux was measured by Universal Photometer. Degradation of AMP and formation of oxidation by-products were monitored by UV absorbance (Schimadzu, UV-1800) and LC-MS/MS instrument (AB SCIEX, 300 QTRAP, USA). Standard solution of AMP was prepared in ultrapure water. Known for its high recovery rates on acidic, basic and neutral analytes and being balanced in terms of hydrophilic–hydrophobic properties, OASIS HLB cartridge was selected and used for AMP analysis [7]. A 99% of recovery was obtained for determination of AMP provided with a linearity over 99% as seen in Quadratic calibration curve (Supplementary Fig. 1). For the parent ion detected at 349 g mol^{-1}, precursor ions were detected to be 106 and 160 g mol^{-1}, respectively (Supplementary Fig. 2).

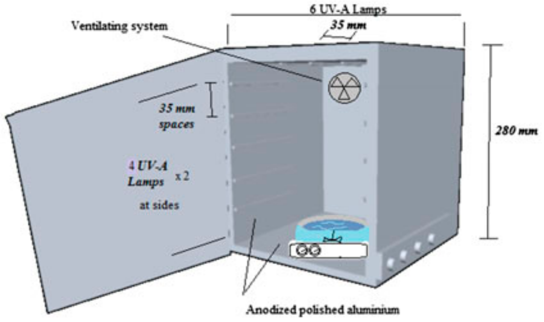

Fig. 2. Schematic view of the illumination reactor and the magnetically stirred vessel inside.

Mineralization was determined by TOC measurement (Schimadzu, 6KVA model)). pH was also monitored (Hach Lange multi parameter) to better evaluate mechanism and efficiency of the photocatalytic oxidation.

2.5. Ecotoxicity tests

Acute toxicity of AMP solutions prior and post-photocatalytic treatment was assessed by freshwater crustacean, *D. magna* according to ISO method 6341 [25]. The pH of the medium was adjusted to 8.0 and the total hardness was 250 mg L^{-1} (as $CaCO_3$). Newborn daphnids (<24 h) were exposed to the samples during 24 and 48 h. Toxicity tests were performed in the dark at 20˚C as four replicates using five daphnids in each vessel with 50-mL sample volume. Toxicity of each sample was assessed by means of immobilization percentage that was determined by dividing total number of immobilized organisms to total number of tested organisms [4,15].

3. Results and discussion

3.1. Effect of pH on TOC removal

The pH of the solution can affect the electrostatic charge in the photocatalytic reaction occurring on TiO_2 surface. pH is an important parameter in the adsorption of ionic organic matter especially on the surface of TiO_2. Therefore, pH is a key factor that determines the photocatalytic oxidation efficiency and the surface charge density of photocatalyst. Thus, the effect of the photocatalytic oxidation of organic matter was examined at three different pH values of 5, 7 and 11 in this study.

Although a lesser than 15–20% removal of target antibiotic was reported by the adsorption mechanism elsewhere [4], neither photolysis nor dark experiments (adsorption) did exhibit any significant removal of degradation or mineralization at studied pH values in this study (data not shown). On the other hand, in accordance with the literature findings, optimum pH was defined to be pH 5 for both initial (50 and

100 mg L^{-1}) AMP concentrations as seen in Fig. 3. At pH 5 conditions, surface interactions are supposed to have occurred between the (+) charged TiO_2 (Zcp of 6,8) and (−) charged AMP (pK_a value of 7.3). In parallel, the active carboxyl groups may have promoted photocatalysis rates with increased rate of direct surface oxidation on TiO_2 [23,26].

3.2. Effect of UV Energy Levels on TOC Removal

TOC removal efficiency evolved to be proportional with the increase in UV energy that may find evidence with the analogous literature findings [4,16,22,23,26]. As shown in Fig. 4, the highest TOC removal efficiency was obtained at the 4.94 mW/cm^2 energy level at the optimized pH value of 5 during oxidation of 50 mg L^{-1} AMP.

A relatively high correlation ($R^2 = 0.866$) was noted between TOC removal rates and UV energy levels (Fig. 5).

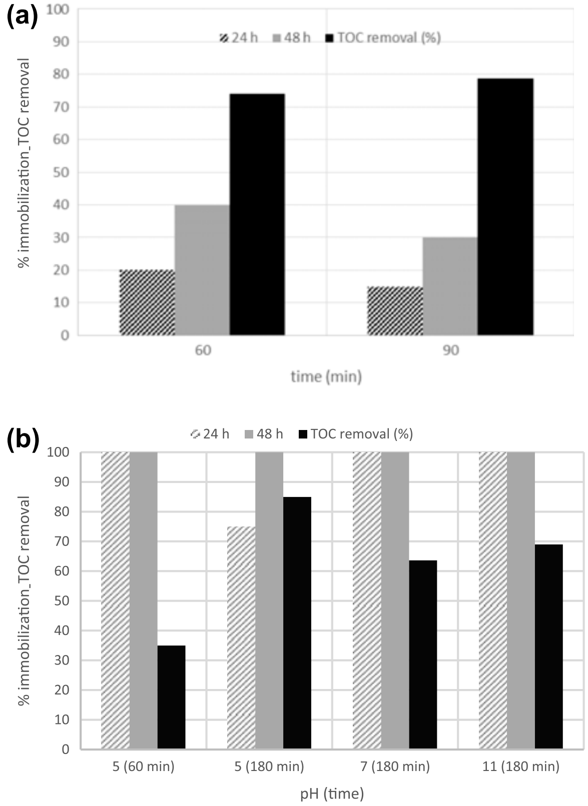

Fig. 3. Effect of pH, process time and initial AMP concentration on TOC removal rates and toxicity evolution (Initial TOC values = 27.4 and 52.36 mg L^{-1} for 50 (a) and 100 mg L^{-1} (b) AMP respectively; $TiO_2 = 0.5$ g L^{-1}; 4.92 mW/cm^2).

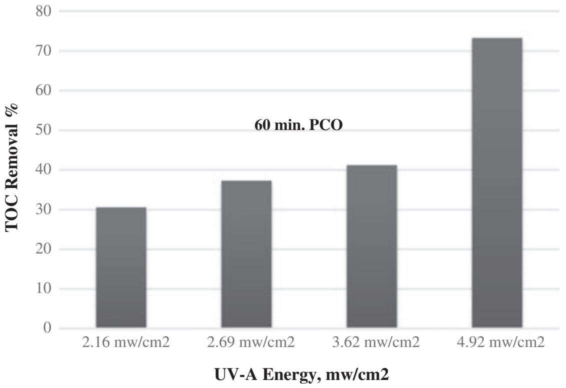

Fig. 4. TOC removal evolution vs. UV-A energy levels, 50 mg L^{-1} initial AMP, 0.5 g L^{-1} TiO_2, pH 5, process time: 60 min).

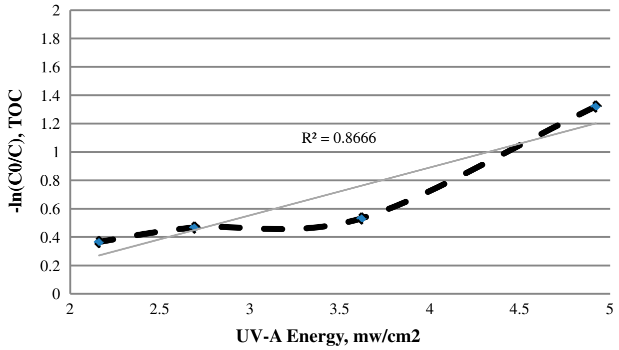

Fig. 5. TOC removal rates correlation with varying UV energy levels at 50 mg L^{-1} AMP, pH 5, 0.5 g L^{-1} TiO_2, during 60 min of photocatalytic oxidation.

3.3. Effect of initial antibiotic concentration on TOC removal

Unit ratio between quantity of catalyst and target compound is important for providing a larger surface area for the adsorption as well as the higher potency of pairing of electrons to produce hydroxyl radicals for a higher oxidation efficiency [22,23]. Thus, lower initial AMP concentration enabled higher removal rates by higher ratio of catalyst/AMP vs. process time run at the maximum UV energy level as the all lamps were switched on (Fig. 3). According to Fig. 3(a), TOC removal increased slightly (4%) while irradiation time was prolonged from 60 to 90 min at pH 5 and 0.5 g L^{-1} TiO_2 dose. This trend was confirmed by a slight toxicity decrease (10%).

When initial AMP concentration was doubled and submitted to 60-min photocatalytic irradiation at pH 5 using the same catalysts dose, around a 30% mineralization rate was obtained (Fig. 3(b)). It was necessary to prolong photocatalysis duration two times to obtain the same level of mineralization at 100 mg L^{-1} initial AMP. It is expected that higher initial AMP concentration should have given rise to increase in oxidative transformation by-products that should cause the increase in toxicity (Fig. 3(a)). Findings of rapid AMP degradation at the lower initial concentrations and reduced toxicity are in accordance with the analogous studies so far [22,23,26].

AMP and most of the penicillin group antibiotics, absorb light below 300-nm wave band [6]. When this band range was focused on, an UV absorbance values during 0–30 min oxidation period was observed for both initial AMP concentrations (Fig. 6).

3.4. Degradation and mineralization of AMP: by-products formation and toxicity

A substantial degradation rate of 50 mg L^{-1} AMP between 0 and 15 min period and a descent continuity between 15 and 30 min were observed during photocatalytic oxidation of AMP using 0.5 g L^{-1} TiO_2 and at pH 5 under 4.92 mW/cm^2 of UV-A energy level (Fig. 7). The increase in toxicity in parallel to degradation revealed that there was occurrence of toxic by-products that stabilized and become less toxic vs. prolonging oxidation time.

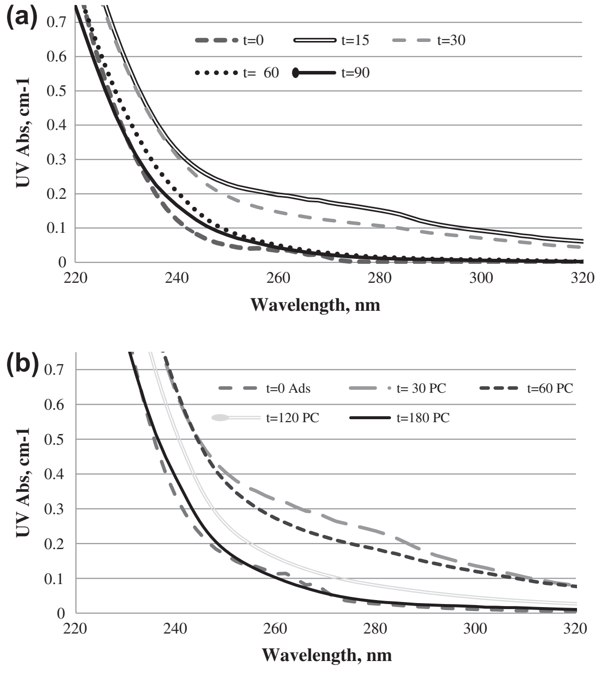

Fig. 6. UV absorbance peaks vs. time for two initial AMP concentrations ((a) 50 mg L^{-1} and (b) 100 mg L^{-1}) at pH 5, 0.5 g L^{-1} TiO_2, 4.92 mW/cm^2 UV-A.

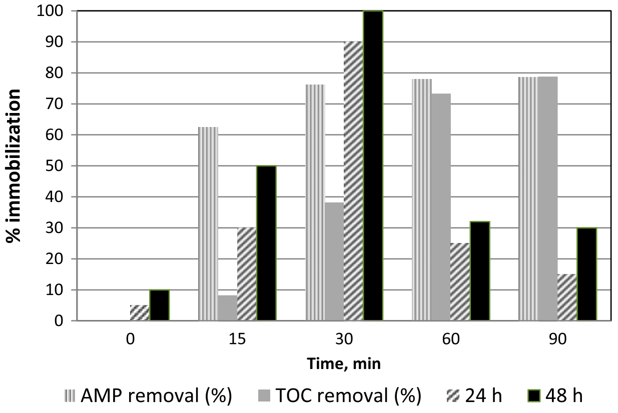

Fig. 7. Time dependent AMP removal, TOC and Toxicity reduction at 50 mg L^{-1} initial AMP concentration, 0,5 g L^{-1} TiO_2, 4.92 mW/cm^2 UV-A and pH 5 conditions.

As highlighted in the Fig. 8(a), the initial 15 min of photocatalysis comprised of an on-going degradation (>80%). The increasing trend in the UV absorbance spectra (Fig. 6) and an equivalent reduction in AMP concentration indicated a substantial photocatalytic

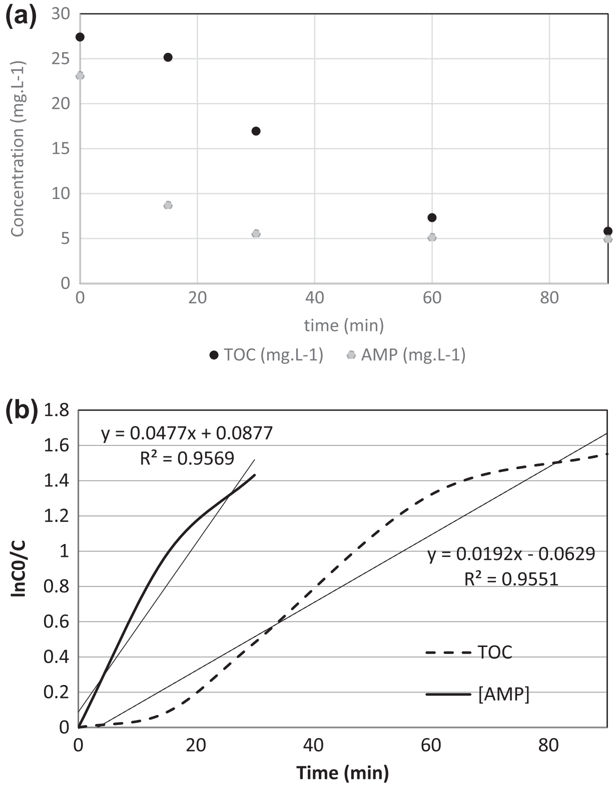

Fig. 8. Degradation of and mineralization of 50 mg L^{-1} AMP (a) and Kinetic analysis of 50 mg L^{-1} AMP (b) at pH 5, 0.5 g L^{-1} TiO_2, 4.92 mW/cm^2 UV-A.

Table 1
Cost evaluation of photocatalytic AMP removal by means of chemical reagents and energy requirement

Energy requirement [13]				
UV-A energy levels (mW/cm^2)	4.92	3.62	2.69	2.16
Reaction rate (k min^{-1})	0.022	0.0088	0.0076	0.0062
Energy requirement ($/kg TOC)	1,882	1,732	1,620	1,590
Chemical Requirement [22]				
	HCl		TiO_2	
Cost estimation ($/kg TOC)	23.9		111	
Total ($/kg TOC)	~135			

degradation and the formation of by-products which influenced UV spectrum, mainly oxidation of an opening ring [26–28]. As shown in Fig. 8(a), a sharper decrease in AMP indicated, as a consequence, the formation of intermediates which exhibited toxic properties as mentioned in Fig. 7. Meanwhile, a strong correlation ($R^2 = 0.956$) was obtained for both TOC and AMP removals (Fig. 8(b)).

3.5. Cost estimation

A cost estimation was carried out in accordance with a recently developed procedure for UV energy consumption estimation based on assessment of electrical energy requirement to efficiently degrade unit mass of organic pollutant according to Eq. (1):

$$\mathrm{EE} = \frac{p \times t \times 1000}{V \times M \times 60 \times (C_\mathrm{i} - C_\mathrm{f})} \quad (1)$$

where p is lamp power in kWh, V is the volume of polluted water in m^{-3}, t is the time required to achieve a 50% mineralization (TOC removal), M is the molecular weight of the target pollutant in g mol^{-1}, C_i and C_f are initial and final concentration of the pollutant in mol L^{-1}. Prices of electricity and the reagents were selected from literature studies on the subject [13,22]. As can be seen in Table 1, it is notable that prolonged irradiation time would not be satisfying neither to achieve complete detoxification nor to promote mineralization. Here, it was defined that more than 3.62 mW/cm^2 of UV-A energy level must be applied for the experimental conditions (0.5 g L^{-1} TiO_2, 50 mg L^{-1} AMP concentration, pH 5) to achieve >50% total mineralization and complete detoxification of treated AMP solution.

For a better cost evaluation considering experimental data-sets, it would also be convenient to provide a reactor configuration and set-up information such as water depth in the reactor as well as distance between UV energy source and the surface of the solution in the reactor [4,16].

4. Conclusion

From the results of this study, the following can be concluded:

1. Photocatalytic removal efficiency of AMP was found to be in accordance with the UV absorbance measurements. TOC removal efficiency was revealed to be proportional to UV energy variation.
2. Level of UV energy is an important parameter to optimize process efficiency. It was found to be in good correlation with the total mineralization and detoxification efficiency of the heterogeneous photocatalytic oxidation system.
3. Cost estimation was assessed considering UV energy level and chemical reagents. It was noted that there was no need to prolong irradiation time to the maximum energy level for satisfying complete detoxification and mineralization.

Supplementary material

The supplementary material for this paper is available online at http://dx.doi.[10.1080/19443994.2016.1155175].

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