Solar photo–Fenton as finishing step for biological treatment of a pharmaceutical wastewater

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Abstract

Remediation of pharmaceutical wastewater, containing nalidixic acid (NXA, 38 mg/L), a quinolone antibacterial agent commonly used in human and veterinary medicine, and characterised as having mainly 725 mg/L of dissolved organic carbon (DOC), 3400 mg/L of COD and NaCl of around 4 g/L, was studied. A prior biodegradability study (Zahn-Wellens test) had demonstrated that the matrix was biodegradable after a rather long biomass adaptation period. After 4 days of treatment in an immobilized biomass reactor (IBR), 96% of the original DOC was removed by the biological treatment, however, more than 50% of NXA was adsorbed on the biomass. As development of chronic toxicity in the IBR is possible after long exposure to NXA, adsorption and biomass stability during continuous exposure to NXA were studied in different cycles for one month. After the biotreatment, the effluent was treated by solar photo-Fenton. Total degradation of NXA and reduction in toxicity were observed. The intermediates formed during degradation by biotreatment and subsequent photo-Fenton were studied by Liquid Chromatography- Time of Flight-Mass Spectrometry.

Keywords: Detoxification, nalidixic acid, immobilised biomass reactor, solar photo-Fenton.

Introduction

Today's extensive use of pharmaceuticals for preventing and treating infectious diseases in both humans and animals has contributed to the appearance of a new class of potentially hazardous organic pollutants. The occurrence and fate of antibiotics in the aquatic environment has been internationally documented *(1, 2, 3, 4, 5)*. Antibiotics are of particular concern, as they can induce bacterial resistance, even at low concentrations *(6, 7, 8, 9)*. In addition, many pharmaceutical substances are often not eliminated by wastewater treatment or biodegraded in the environment, as described in recent reviews *(10, 11, 12, 13)*.

It has been demonstrated that the use of advanced oxidation processes (AOPs) can break down a wide range of pharmaceutical molecules due to the on-site generation of sufficient hydroxyl radicals ($^{\bullet}OH$), a highly potent, extremely reactive chemical oxidant, to oxidize these compounds and purify water. Light-driven AOPs produce hydroxyl radicals by combining oxidants such as ozone or H_2O_2 with highly energetic UV-C radiation or in the presence of catalysts such as metal ions or semiconductors and irradiation in the UV, or even in the visible range. Among the AOPs, photo-Fenton has gained increasing attention due to its simplicity and the possibility of using sunlight for the reaction, considerably lowering the operating cost *(14)*. Its application for the degradation of pharmaceuticals, such as antibiotics, hormones, analgesics, anti-inflammatory drugs and others, has recently been reported *(15, 16, 17)*.

The efficacy of AOPs in treating a wastewater is strongly dependent on (i) the composition and concentration of the wastewater in question and (ii) the treatment target, so the heavier the pollutant load and the more removal is required, the stronger the treatment conditions must be. From this point of view, treatment performance can be enhanced by the application of AOPs as a pre-treatment stage to enhance biodegradability and reduce toxicity followed by biological post-treatment. This concept, which has attracted much attention over the past several years *(18,*

19), is relatively straightforward and based on (i) biological treatment being less expensive and more environmentally friendly than any other destructive treatment, and (ii) complete mineralisation by AOPs leads to excessive treatment cost since the highly oxidized end-products tend to be resistant to total chemical oxidation. In previous studies, it has been observed that the products remaining after AOP treatment of pharmaceuticals are biodegradable *(20, 21, 22, 23)*, and therefore more suitable for biological treatment. But studies involving real wastewater treatment are scarce, and still scarcer those taking into account that wastewater often contains a significant number of biodegradable compounds along with those that are biorecalcitrant.

The aim of this study was to find the best way of treating wastewater containing NXA, a synthetic antibacterial agent frequently used in the treatment of urinary tract infections involving Gram-negative organisms *(24)*, by combining an AOP and a biotreatment. A pilot solar photo-Fenton -plant and an immobilised biomass reactor (IBR), were used for the study. The work also involved a detailed liquid chromatography-time of flight-mass spectrometry (LC-TOF-MS) study of intermediates formed during both treatments.

Experimental

Chemicals

The NXA standard was provided by Fluka. Photo-Fenton experiments were performed using $FeSO_4 \cdot 7H_2O$ and reagent-grade H_2O_2 (30% w/w). The pharmaceutical wastewater had a pH of 3.98, 725 mg/L of DOC, 3400 mg/L of COD, 0.4 g/L of TSS, 38 mg/L of NXA and inorganic ions (Cl^- = 2.7 g/L, Na^+ = 1.8 g/L, PO_4^{3-} = 0.018 g/L and <0.1 mg/L of NH_4^+ and NO_3^-). Other organics present in the pharmaceutical wastewater were analysed by LC-TOF-MS (Table 1). The actual treatment procedure includes blending the wastewater with other water and the combined stream is disposed of in a central biotreatment plant. NXA was found to cause serious problems in such a centralized treatment plant.

Analytical methods

An Agilent Series 1100 HPLC system with a UV-DAD detector (λ = 254nm), C-18 column (Phenomenex LUNA 5 µm, 3 mm x 150 mm) and methanol/formic acid (25mM) 50/50 mobile phase were used to monitor NXA concentration during degradation. Dissolved organic carbon (DOC) was monitored by a total organic carbon analyser (Shimadzu-5050A TOC analyser). NH_4^+, Na^+, NO_3^- and Cl^- were monitored by ion chromatography (Dionex DX120 for cations and Dionex DX600 for anions). Chemical oxygen demand (COD) was measured with Merck® Spectroquant kits. Total iron concentration was monitored by colorimetric determination with 1,10-phenanthroline, according to ISO6332. Fe(III) was reduced with ascorbic acid to determine the total amount of iron. Hydrogen peroxide was analyzed by a spectrophotometric method using ammonium metavanadate *(25)*.

Solid-phase extraction using Oasis™ HLB cartridges was applied first to reduce the salt content in the matrix before chromatographic analysis, and to improve intermediate detectability. Intermediates were studied by Liquid Chromatography- Time of Flight-Mass spectrometry using positive-ion mode electrospray ionization in positive mode, LC-TOF-MS (ESI+). The HPLC System (Agilent Technologies Series 1100) was equipped with a ZORBAX, SB-C18 analytical column (5 µm, 3 mm x 250 mm). The mobile phase was a mixture of acetonitrile acidified by 0.1% formic acid (A) and water acidified by 0.1% formic acid (B) at a flow rate of 0.4 mL min^{-1}. A linear gradient progressed from 10% A (initial conditions) to 100% A in 30 minutes, and then remained at 100% A for 5 minutes. A connected TOF mass spectrometer (Agilent Technologies) was equipped with an electrospray interface operating with a capillary voltage of 4000 V, nebulizer 40 psi g, drying gas 9 L min^{-1}, gas temperature 300ºC; skimmer voltage 190 V, octapole DC1, 37.5 V, and octapole rf 250 V. Data were processed with Applied Biosystem/MDS-SCIEX Analyst QS software with Agilent MSD TOF specific

accurate mass application- software. Possible elemental ion composition was assigned with a maximum deviation of 6.6 ppm.

Biotreatment

The biological reactor was an immobilized biomass reactor (IBR) consisting of a 160-L flat-bottom tank filled with 90-95 L of polypropylene Pall Rings (15 mm nominal diameter) colonized by activated sludge from a conventional wastewater treatment plant (WWTP) in Almeria (southeast Spain). The system is composed of a 100-L conditioner tank with a pH control system connected to the IBR by a recirculation pump (500 L/h). Dissolved oxygen, pH and temperature were automatically measured and registered. The bioreactor was operated in batch mode.

The biological reactor was started up and adapted, starting with immobilization of sludge on the rings, which took two days (total suspended solids = 0 g/L). After this, the system was maintained by controlled addition of glucose and ammonium chloride, keeping the carbon/nitrogen ratio at 100/20. The next step was to adapt the sludge to high NaCl content (approximately 5 g/L). Total suspended solids, DOC, pH, and nitrogen concentration (as NH_4^+ and NO_3) were analysed throughout IBR adaptation.

Solar photo-Fenton

All solar photo-Fenton experiments were performed in a compound parabolic collector (CPC) solar photocatalytic pilot plant composed of two modules of twelve Pyrex glass tubes mounted on a fixed platform tilted 37º (local latitude), with a total area of 3 m^2 and 40-L volume (22 L irradiated) *(26)*.

At the beginning of all the photo-Fenton experiments, the wastewater was added to the photoreactor. Then the pH was adjusted to 2.6-2.8 with H_2SO_4 (2 N). Afterwards, iron salt was

added ($FeSO_4.7H_2O$, 20 mg/L of Fe^{2+}) and mixed. Finally, the first dose of H_2O_2 (300 mg/L) was added and the reactor uncovered. Samples were taken at time intervals during the experiments to evaluate degradation and H_2O_2 concentration was kept in the range of 200- 400 mg/L during the experiments.

Solar ultraviolet radiation was measured and recorded continuously by a global UV radiometer (KIPP&ZONEN, model CUV 4). With Equation 1 *(27)*, combination of the data from several days' experiments and their comparison with other photocatalytic experiments is possible, where t_n is the experimental time for each sample, UV is the average solar ultraviolet radiation measured during Δt_n, and t_{30W} is a "normalized illumination time". In this case, time refers to a constant solar UV power of 30 $W.m^{-2}$ (typical solar UV power on a perfectly sunny day around noon). V_T is the total volume of the water loaded in the pilot plant (40 L), and V_i is the total irradiated volume (22.0 L).

$$t_{30W,n} = t_{30W,n-1} + \Delta t_n \frac{UV}{30}\frac{V_i}{V_T}; \quad \Delta t_n = t_n - t_{n-1} \qquad (1)$$

Toxicity and biodegradability assays

Toxicity of the original pharmaceutical wastewater and final samples of each process was evaluated using a commercial luminescence inhibition assay marketed as Biofix®Lumi-10 (*Vibrio fischeri*). Sample inhibition effect on the bacteria was analysed by measuring the drop in light emission after contact periods of 30 minutes compared to a toxicant-free control (ISO 11348-3). Toxicity of the same samples was also measured using the crustacean *Daphnia magna* (Cladocera) with a commercially available kit (Daphtoxkit Creasel). The toxicity studies were performed in accordance with test conditions prescribed by OECD Guideline 202 and ISO 6341.

The biodegradability of the original pharmaceutical wastewater was found by a 28-day Zahn-Wellens (Z-W) test (an adaptation of the EC protocol, Directive 88/303/EEC) done at 20-25ºC under diffuse illumination *(28)*.

Results and discussion

The main wastewater parameters were described above in the Experimental section. The Z-W test showed significant biodegradability (around 80%), but only after 15 days, and NXA concentration remained constant, demonstrating that NXA is biorecalcitrant and cannot be degraded by conventional biotreatment. The Z-W test showed that although the most of the organic content of the wastewater could be biodegraded, the biomass required a long adaptation period and NXA persisted. This demonstrated that a biological system could treat the matrix studied, but not NXA, which is not biodegradable and should be removed afterwards by an AOP.

Evaluation of combined biological and photo-Fenton treatment

The IBR was emptied and filled with pharmaceutical wastewater with pH previously adjusted to around pH = 7, and maintained between 7 and 8 by an IBR pH control system. DOC and NXA concentrations were monitored throughout the biological treatment. After 4 days, 96% of the initial DOC was removed, demonstrating that biomass immobilization was successful. On the first day, NXA concentration was reduced to 14 mg/L and remained constant until the end of the process. This sharp drop in NXA could only be related to the adsorption of the compound on the IBR (biomass or 15-mm polypropylene Pall Rings).

NXA is a very weak organic acid with a pKa of 5.95 that may be present in both neutral and anionic forms in the neutral pH range. Therefore, the adsorption capacity of this compound also varies according to the pH. Lorphensri et al. *(29)* studied the adsorption and transport of some pharmaceuticals, including NXA, in low-organic-content aquifer sand. NXA showed

much stronger adsorption in low-organic aquifer sand than expected based on its hydrophobicity. The adsorption of NXA was also observed to be highly dependent on pH, which further supports electrostatic forces as dominant in the sorption *(29)*. The pH in the IBR was around 7 throughout the process, with NXA solubility around 300 mg/L and log K_{ow} 0.4 (K_{ow}, octanol-water partition constant). Stackelberg et al. *(30)* also studied removal of the pharmaceutical in conventional drinking-water-treatment processes. Hydrophobic compounds, such as NXA, are generally detected at elevated concentrations in dried-solid samples and are not present at measurable concentrations in finished-water samples. As there was no inorganic N in the wastewater matrix, nitrogen as ammonium chloride was added to satisfy the need for a constant C:N ratio (100:20) in the aerobic biological systems. Inorganic nitrogen (NH_4^+ and NO_3^-) was monitored during the biotreatment, however no nitrification was observed.

After the biological treatment, the partially biotreated wastewater was treated by photo-Fenton (Figure 1). Two degradation studies were carried out. In the first, the biotreatment effluent (NXA 13 mg/L and DOC 22 mg/L) was treated directly. In the second study, the biotreated wastewater was enriched by adding NXA standard until 38 mg/L,, simulating absence of IBR adsorption of NXA after biomass saturation. The other parameters, common to both studies, were 20 mg/L of iron, pH = 2.7 and H_2O_2 concentration kept between 200 and 400 mg/L. In both cases, NXA was completely degraded at around 25 minutes of illumination time and 12 mM of H_2O_2 consumed, reaching a DOC of around 20 mg/L, which is low enough for disposal. In both experiments, after 50 minutes of illumination time, the DOC reaction rate decreased drastically, however, H_2O_2 consumption continued. The overall efficiency of the combined IBR+solar photo-Fenton treatment in terms of elimination of DOC was over 97%. Biorefractory compounds are very often not so toxic to activated sludge,, and biodegradation prior to AOP may be successful, avoiding long treatment times and high chemical consumption, the main drawbacks of AOP treatments. In these cases, AOP treatment is more suitable as a

polishing step than as a pretreatment. It should be mentioned that direct photo-Fenton treatment of this wastewater required around 3 hours of treatment time and 85 mM of H_2O_2 to completely degrade NXA, and 600 mg/L of DOC still remained after NXA had completely disappeared (*31*).

On the other hand, the presence of Cl^- in the wastewater matrix affects the photo-Fenton process, as Cl^- acts as a hydroxyl radical scavenger. Less reactive species, such as chlorine atoms ($Cl^{\bullet}$) and dichloride anion radicals ($Cl_2^{\bullet-}$), are generated by Reactions 2-4.

$$Cl^- + HO^{\bullet} \rightarrow ClOH^{\bullet-} \quad (2)$$

$$ClOH^{\bullet-} + H^+ \rightarrow Cl^{\bullet} + H_2O \quad (3)$$

$$Cl^{\bullet} + Cl^- \rightarrow Cl_2^{\bullet-} \quad (4)$$

The negative effect of Cl^- on photo-Fenton efficiency is also related to the formation of iron(III)-chloro complexes, which inhibit the formation of iron(III)-hydroperoxide complexes, active species in photo-Fenton process. Similarly, by Reactions 5-6, chloride ions also make heavier consumption of hydrogen peroxide necessary for the same mineralisation. De Laat and Le *(32)* conclude that over 100 mM of chloride inhibits formation of iron(III)-hydroperoxide complexes and slows down the reaction rate. Therefore, the photo-Fenton NXA mineralisation rate in saline water could be expected to be inhibited. Active chlorine compounds formed could have other effects, such as the formation of chlorinated intermediates, described in previous studies (*33*). However, in this study, assuming that they were formed, they were not detected by LC-TOF-MS.

$$Cl^{\bullet} + H_2O_2 \rightarrow HO_2^{\bullet} + Cl^- + H^+ \quad (5)$$

$$Cl_2^{\bullet-} + H_2O_2 \rightarrow HO_2^{\bullet} + 2Cl^- + H^+ \quad (6)$$

Toxicity was monitored using two different organisms, *Vibrio fischeri* and *Daphnia magna*. *Vibrio fischeri* showed high toxicity (97% inhibition) in the first wastewater sample, but

did not show any after biological treatment, and at the end of photo-Fenton, inhibition was 59%. Toxicity could be related to the presence of organic acids characteristic of the last stages of the oxidation process. *Daphnia magna* bioassay demonstrated very high toxicity in the original matrix (all daphnids dead after 24 h of culturing) and the biotreated sample did not show any toxicity at all. At the end of the photo-Fenton treatment, the sample was not acutely toxic to *Daphnia magna*, which showed mobility of 95% after 48 h of culture.

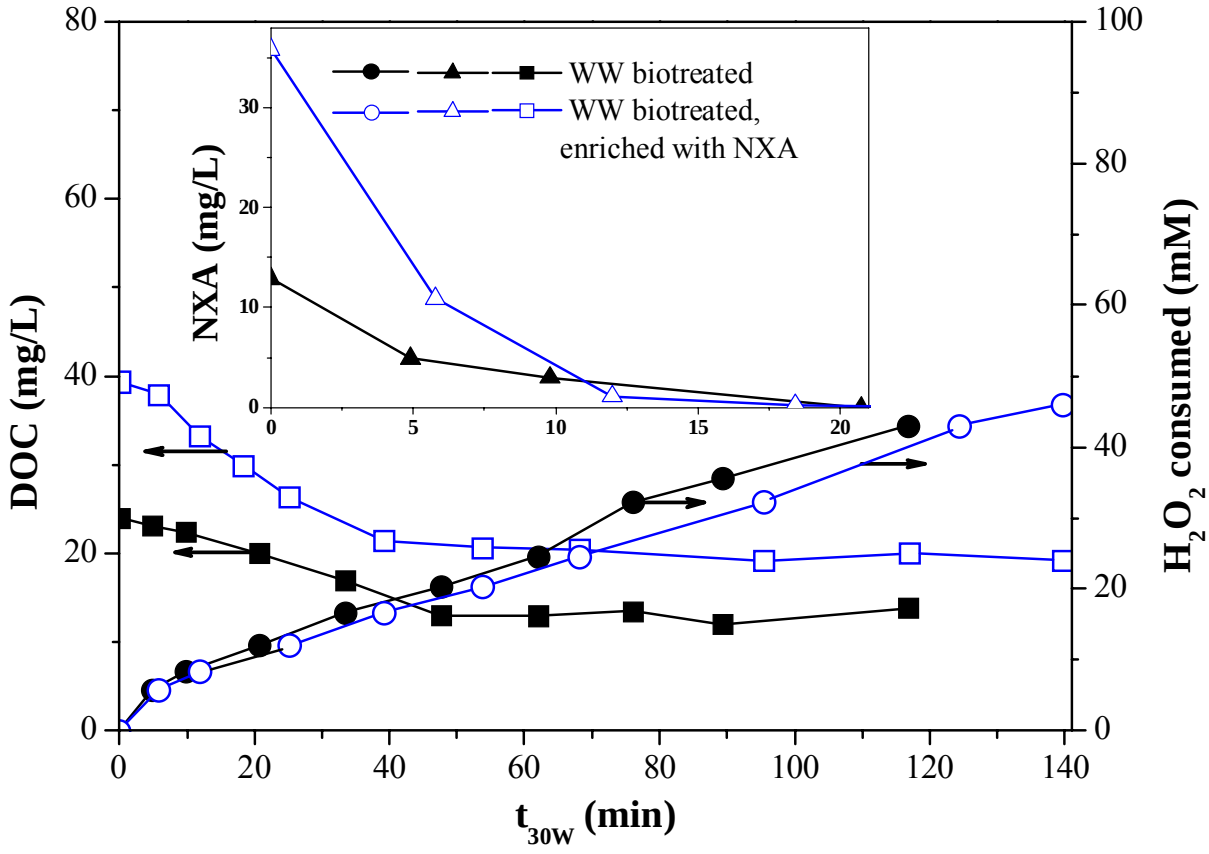

Figure 1: DOC reduction, NXA degradation (inset) and H_2O_2 consumption during photo-Fenton treatment: Fe =20 mg/L , pH_0 = 2.7, [H_2O_2] = 200-400 mg/L.

Evaluation of continuous biomass exposure to NXA

As NXA is an antibacterial agent, chronic toxicity could develop in the IBR after long exposure. Therefore, biomass stability and adsorption studies were performed during continuous exposure to NXA. To better evaluate this effect, instead of real wastewater, synthetic

wastewater containing NXA and a similar DOC concentration was prepared. This procedure was intended to evaluate only the effect of NXA, avoiding any interference from unknown compounds present in wastewater. The study was divided in five steps with addition of a synthetic influent (S.I.; DOC around 600 mg/L) and 45 mg/L of NXA in each (Figure 2). The chemical composition of the synthetic influent adapted from OECD was 960 mg/L of peptone, 660 mg/L of meat extract, 180 mg/L of urea, 28 mg/L of K_2HPO_4, 4 mg/L of $CaCl_2.2H_2O$ and 2 mg/L of $Mg_2SO_4.7H_2O$ *(34)*.

The reactor was emptied between runs. The 5th run was maintained for seven days to observe the progress of the parameters studied during longer periods and determine any change in NXA concentration with low DOC or biomass death (DOC should increase) due to chronic toxicity, etc. After that, the system was emptied and filled with S.I. without NXA (6th cycle) to evaluate possible desorption. In all of the steps, significant DOC reduction was observed (>90% mineralisation), while the pH remained between 6.8-8.0.

NXA concentration was only reduced in the first run and in the first measurement of the 2nd run due to adsorption on the biomass, after which it returns to the original concentration after just 26 hours of treatment. However, in the 3rd and 4th runs, pollutant concentration increased. This means that the biomass adsorption rate decreased over exposure time to NXA. Indeed, during runs 3 and 4, NXA adsorbed during the 1st run (around 25 mg/L) was released back into the water. During run 5 there was again slight adsorption. Finally, during run 6 (S.I. without NXA) no desorption and only traces of NXA were detected. As commented above, NXA is a very weak organic acid that may be present in both neutral and anionic forms in the neutral pH range. Therefore, the adsorption capacity of this compound may also vary with pH. As pH during the biotreatment varied between 6.8 and 8 and the composition of the water also varied due to DOC biodegradation, NXA adsorption/desorption phenomena did not follow any predictable pattern.

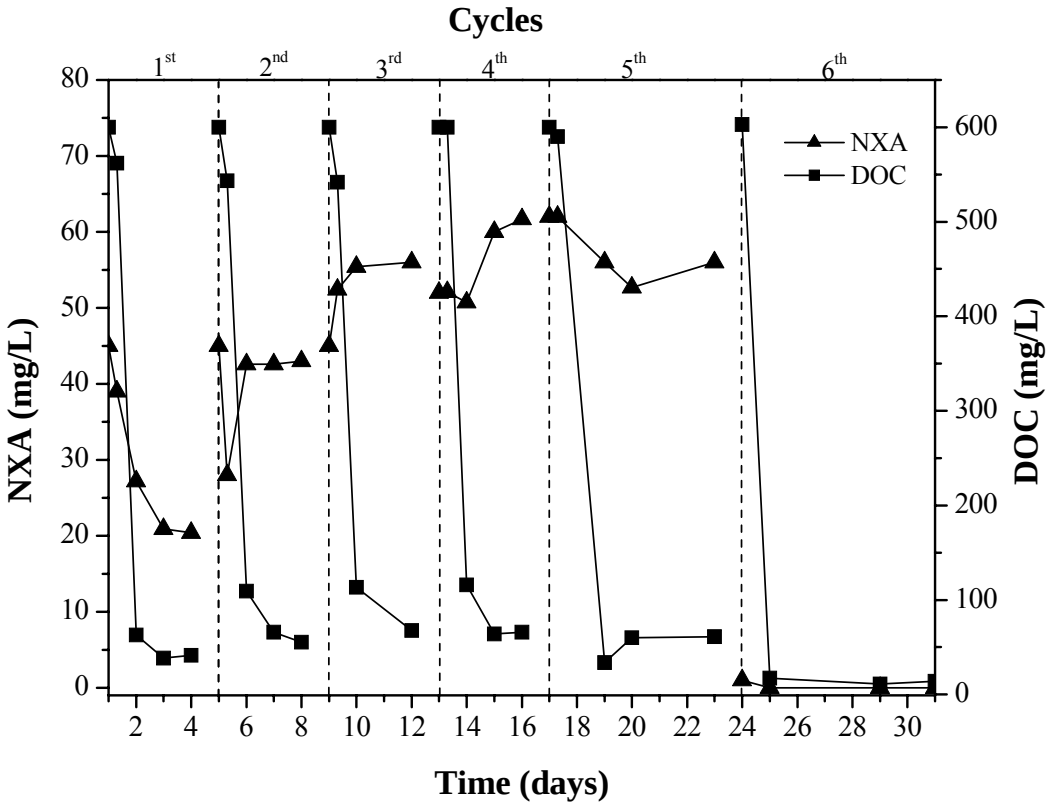

Figure 2: Study of biomass stability and adsorption in IBR during exposure to NXA along 5 runs in batch mode using S.I. and NXA_0 = 45 mg/L. 6th run was only with S.I.

Monitoring of inorganic nitrogen (NO_3^- and NH_4^+) provides more information about nitrification. The first three steps showed a high NO_3^- concentration at the end of the run (between 415 and 530 mg/L), while the concentration of ammonia decreased. During the last steps, nitrification was drastically reduced as the increase in nitrate was insignificant compared to the original concentration of ammonia. This can be interpreted as a probably chronic toxic effect of NXA accumulation on the nitrifying bacteria population in the biological system. When the system was maintained only with S.I., nitrification slowly started up again (300 mg/L of NO_3^- and <0.1 mg/L of NH_4^+ at the end of the 6th run), demonstrating that the nitrifying microorganisms were reactivated.

Evaluation of the combined treatment by LC-TOF-MS

The intermediates formed during the different degradation steps were studied by LC-TOF-MS (ESI+). The elemental composition proposed for the molecules of interest is based on its accurate mass measurements. The efficacy of this analytical technique in the identification of unknown compounds has been widely demonstrated in previous work *(35, 36)*. In this case, 23 different compounds could be identified. Analytical information provided by the LC-TOF-MS analyses is included in Table 1.

Table 1: Accurate mass measurements found by LC-TOF-MS (ESI+) for raw wastewater and wastewater after biotreatment. DBE = double bond equivalent.

Raw wastewater						
Compound	**R_t**	**Formula**	**Experimental mass (m/z)**	**Calculated mass (m/z)**	**ppm Error**	**DBE**
NXA	34.0	$C_{12}H_{12}N_2O_3Na$	255.0744	255.074	1.5	7.5
		$C_{12}H_{13}N_2O_3$	233.0929	233.092	3.5	7.5
		$C_{12}H_{11}N_2O_2$	215.0826	215.0831	-2.4	8.5
		$C_{10}H_7N_2O_2$	187.0506	187.0502	2.1	8.5
P1	33.0	$C_{11}H_{13}N_2O$	189.1027	189.1022	2.4	6.5
		$C_9H_9N_2O$	161.0715	161.0709	3.4	6.5
P2	33.5	$C_{14}H_{19}N_2O_2$	247.1458	247.1441	6.8	6.5
		$C_{13}H_{15}N_2O$	215.1165	215.1178	6.4	7.5
P3	31.4	$C_{12}H_{12}N_2O_4Na$	271.0609	271.0689	0.2	7.5
		$C_{12}H_{13}N_2O_4$	249.0874	249.0669	1.6	7.5
		$C_{12}H_{11}N_2O_3$	231.077	231.0764	2.5	8.5
		$C_{10}H_7N_2O_3$	203.0454	203.0451	1.3	8.5
P4	28.0	$C_{12}H_{15}N_2O_2$	219.1134	219.1128	2.7	6.5
		$C_{12}H_{13}N_2O$	201.1031	201.1022	4.7	7.5
		$C_{10}H_9N_2O$	173.0711	173.0709	0.9	7.5
P5	26.0	$C_{12}H_{12}N_2O_4Na$	271.0609	271.0689	0.2	7.5
		$C_{12}H_{13}N_2O_4$	249.0874	249.0669	1.6	7.5
		$C_{12}H_{11}N_2O_3$	231.0773	231.0764	3.8	8.5
		$C_{10}H_7N_2O_2$	187.0507	187.0502	2.6	8.5
P6	23.6	$C_{12}H_{11}N_2O_2$	215.0818	215.0815	1.3	8.5
		$C_{11}H_{11}N_2O$	187.0863	187.0865	-1.5	7.5
P7	22.8	$C_{11}H_{13}N_2O_2$	205.0976	205.0971	2.1	6.5
		$C_9H_9N_2O_2$	177.0667	177.0658	4.7	6.5
		$C_9H_7N_2O$	159.0558	159.0552	3.2	7.5
P8	20.2	$C_9H_{13}N_2O$	165.1025	165.1022	1.5	4.5
		$C_7H_9N_2O$	137.0707	137.0709	-1.7	4.5
P9	20.0	$C_{11}H_{13}N_2O_2$	205.0978	205.0971	3.1	6.5
		$C_{11}H_{11}N_2O$	187.0872	187.0865	3.2	7.5
P10	19.7	$C_{10}H_{13}N_2O_3$	209.0922	209.092	0.6	5.5
		$C_8H_9N_2O$	149.0709	149.0709	-0.2	5.5
P11	19.4	$C_{12}H_{11}N_2O_3$	231.0766	231.7764	0.7	8.5

P12	18.7	$C_{11}H_{15}N_2O_3$	223.1078	223.1077	0.3	5.5
		$C_9H_{11}N_2O$	163.0862	163.0865	-2.3	5.5
P13	16.5	$C_{11}H_{17}N_2O_2$	209.1285	209.1284	0.2	4.5
P14	15.2	$C_{11}H_{13}N_2O$	189.1027	189.1022	2.4	6.5
P15	14.9	$C_9H_{13}N_2O_2$	181.0977	181.0971	3.0	4.5
		$C_9H_{11}N_2O$	163.0867	163.0865	0.6	5.5
		$C_7H_7N_2O$	135.0554	135.0552	0.8	5.5
P16	8.5	$C_{11}H_{15}N_2O_2$	207.1129	207.1128	0.4	5.5
		$C_{11}H_{13}N_2O$	189.1022	189.1020	-1.2	6.3
		$C_9H_{11}N_2O$	163.0861	163.0865	-3.0	5.5
P17	6.2	$C_9H_{15}N_2O$	167.1178	167.1178	-0.5	3.5
P18	6.1/	$C_6H_9N_2$	109.0761	109.076	0.6	3.5
	8.3	C_6H_6N	92.0497	92.0494	2.4	4.5
Wastewater after biotreatment						
Compound	**R_t**	**Formula**	**Experimental mass (m/z)**	**Calculated mass (m/z)**	**ppm Error**	**DBE**
P19	30.5	$C_{11}H_{11}N_2O_3$	219.0767	219.0764	1.2	7.5
		$C_{11}H_9N_2O_2$	201.0659	201.0658	0.2	8.5
P20	27.0	$C_{11}H_{11}N_2O_4$	235.0729	235.0713	6.6	7.5
		$C_{11}H_9N_2O_3$	217.0610	217.0607	1.0	8.5
P21	26.7	$C_{12}H_{11}N_2O_3$	231.0765	231.0764	0.3	8.5
		$C_{12}H_9N_2O_2$	213.0661	213.0658	1.1	9.5
P22	26.1	$C_{10}H_9N_2O_3$	205.0609	205.0607	0.6	7.5
		$C_{10}H_7N_2O_2$	187.0503	187.0502	0.5	8.5
P23	22.6	$C_{11}H_{11}N_2O_3$	219.0768	219.0764	1.7	7.5

The industrial wastewater had been characterised previously to determine its main organic components and their behaviour after biological and photo-Fenton treatments. Together with NXA, which was the main constituent of the wastewater effluent, a second compound was present in similar abundance (P1, see Figure 3), which was identified as l-ethyl-l,4 dihydro-7-methyl-4-oxo-1,8-naphthyridine, a decarboxylated form of NXA, which has also been described as a product of NXA photolysis and thermolysis *(37)*. An isobaric compound (P14) was also identified at a different retention time (15,2 min), which could correspond with a positional isomer of P1, probably generated during the synthesis of NXA (structure not proposed).

Other lower intensity peaks were present in the chromatogram (see Figure 3), showing the presence of a complex mixture of NXA derivatives. The most relevant were identified and their structures are shown in Figure 4. They may correspond to previous stages of NXA

synthesis or to degradation products generated in the wastewater during storage. The compound labelled P3 was identified as 7-hydroxymethyl-NXA, which has been described as the primary NXA metabolite. This compound exhibits at least in vitro anti-bacterial activity identical in spectrum and potency to that of the parent compound *(38, 39, 40)*.

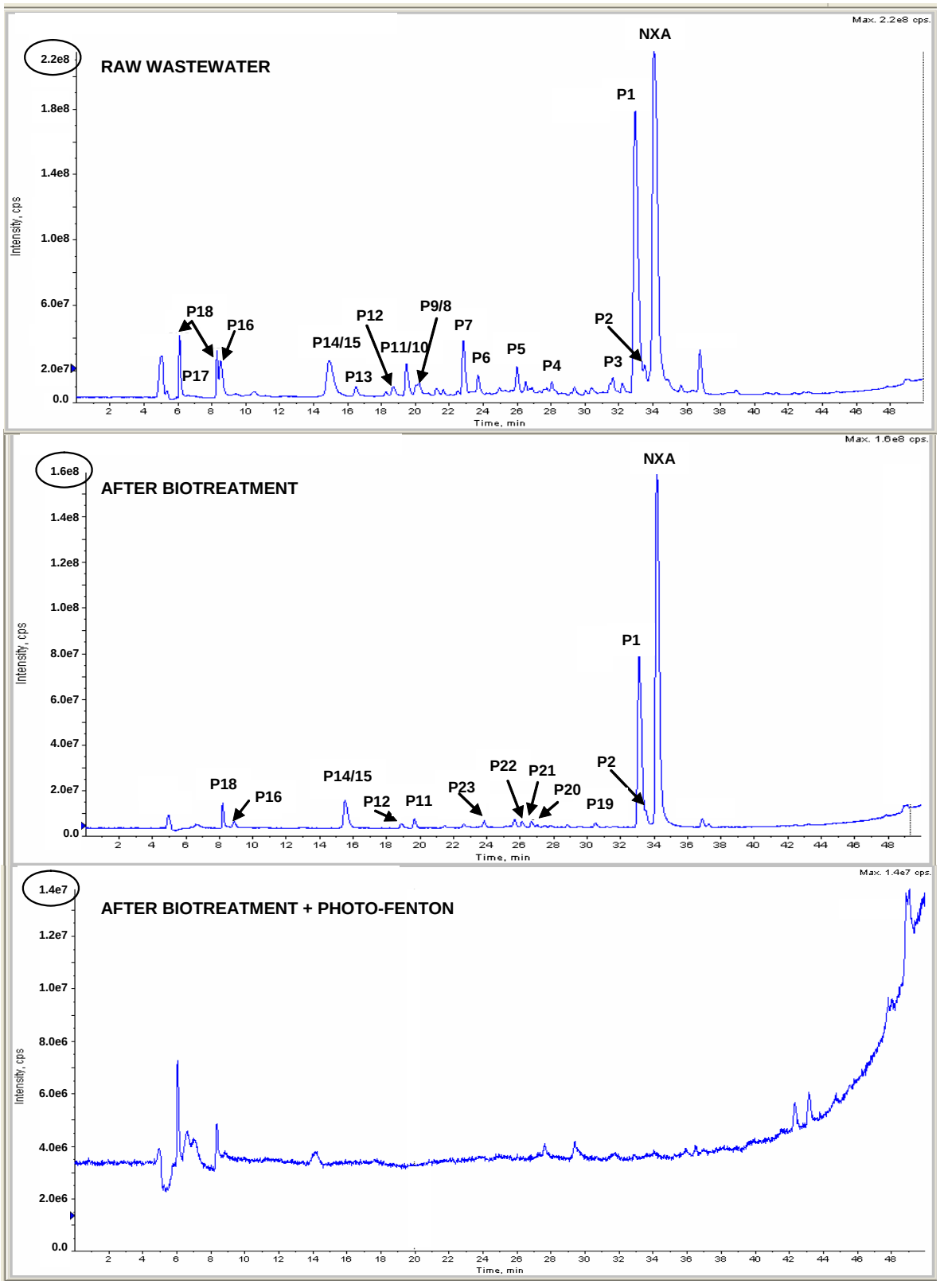

Figure 3: LC-TOF-MS chromatograms obtained by the analysis of a wastewater sample before and after the application of biological and photo-Fenton treatments.

After biotreatment, compounds originally present in the wastewater were completely degraded (P3-P10, P13, P17), or their concentrations dropped as observed in Figure 3 (see

intensity scale). NXA and P1 still remained the main components of the mixture, and a new group of compounds (P19-P23) were identified. The absence of commercial standards for the intermediates identified makes their quantitative evaluation impossible, and the material balance could therefore not be closed.

Only after photo-Fenton treatment, were NXA and analogous compounds totally eliminated, and no further intermediates detected (Figure 3). Residual DOC measured is thus assigned to the presence of organic acids characteristic of the last stages of the oxidative process. This biotreatment, coupled with photo-Fenton, degraded the recalcitrant antibiotic (NXA) originally present in the matrix, and eliminated the by-products generated during biotreatment, demonstrating that photo-Fenton applied to finish off biological treatment of pharmaceutical wastewater is a useful approach.

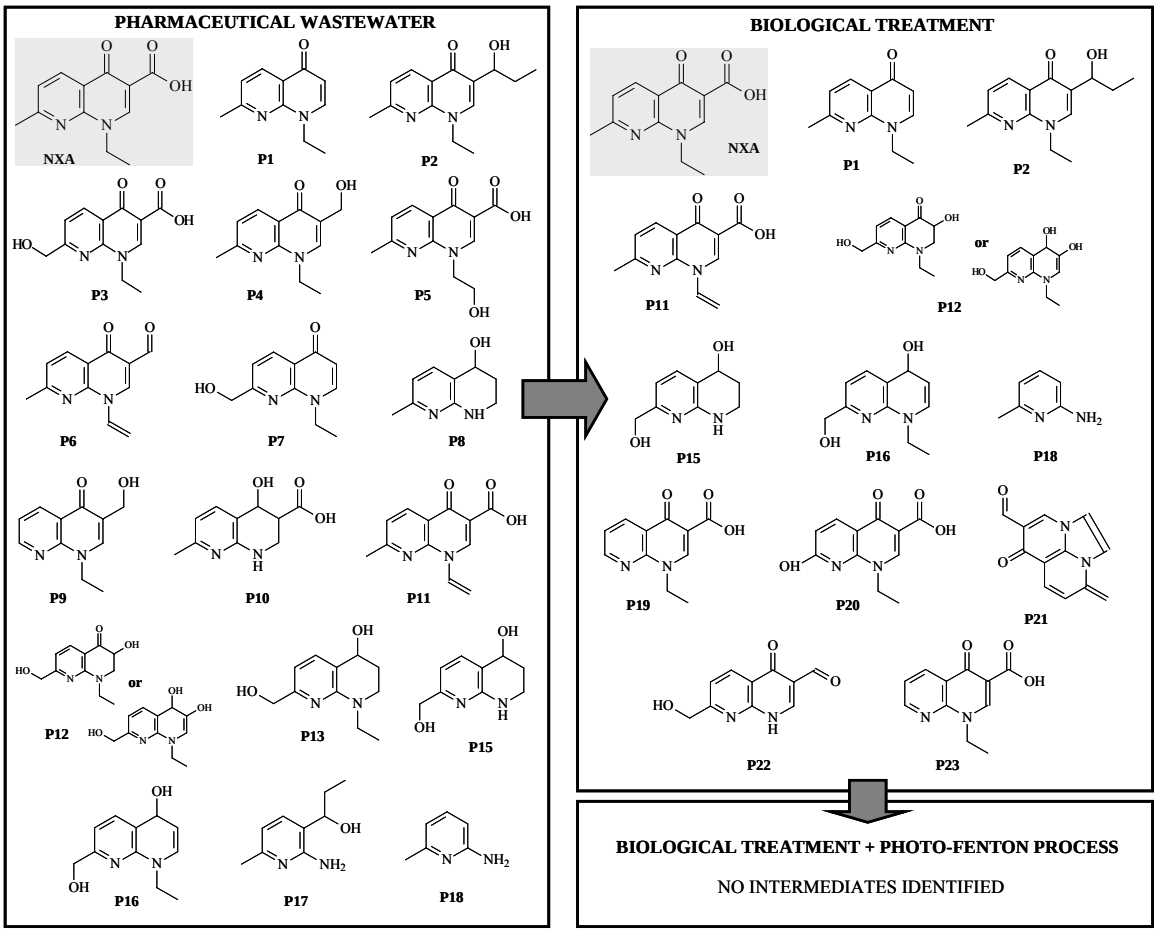

Figure 4: Chemical structures proposed for the intermediates identified by LC-TOF-MS.

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