Decontamination industrial pharmaceutical wastewater by combining solar photo–Fenton and biological treatment

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Abstract

Characterization and treatment of a real pharmaceutical wastewater containing 775 mg dissolved organic carbon per liter by a solar photo-Fenton/biotreatment was studied. There were also many inorganic compounds present in the matrix. The most important chemical in this wastewater was nalidixic acid (45 mg/L), an antibiotic pertaining to the quinolone group. A Zahn-Wellens test demonstrated that the real bulk organic content of the wastewater was biodegradable, but only after long biomass adaptation; however, the nalidixic acid concentration remained constant, showing that it cannot be biodegraded. An alternative is chemical oxidation (photo-Fenton process) first to enhance biodegradability, followed by a biological treatment (Immobilized Biomass Reactor-IBR). In this case, two studies of photo-Fenton treatment of the real wastewater were performed, one with an excess of H_2O_2 (kinetic study) and another with controlled H_2O_2 dosing (biodegradability and toxicity studies). In the kinetic study, nalidixic acid completely disappeared after 190 minutes. In the other experiment with controlled H_2O_2, nalidixic acid degradation was complete at 66 mM of H_2O_2 consumed. Biodegradability and toxicity bioassays showed that photo-Fenton should be performed until total degradation of nalidixic acid before coupling a biological treatment. Analysis of the average oxidation state (AOS) demonstrated the formation of more oxidized intermediates. With this information, the photo-Fenton treatment time (190 min) and H_2O_2 dose (66 mM) necessary for adequate biodegradability of the wastewater could be determined. An IBR operated in batch mode was able to reduce the remaining DOC to less than 35 mg/L. Ammonium consumption and NO_3^- generation demonstrated that nitrification was also attained in the IBR. Overall DOC degradation efficiency of the combined photo-Fenton and biological treatment was over 9597%, of which 33% correspond to the solar photochemical process and 62% to the biological treatment.

Keywords: immobilized biomass reactor, nalidixic acid, photo-Fenton, solar photocatalysis

1. Introduction

Industrial wastewater is often polluted by toxic or nonbiodegradable organic compounds. Special attention currently focuses on pharmaceuticals (Joss et al., 2005; 2006). Their common consumption in human and veterinary medicine generates a diverse range of residual pollutants (pharmaceuticals + metabolites) that reach the aquatic environment through wastewater (Jones, 2001; Heberer., 2002). Antibiotics are of particular concern, as they can induce bacterial resistance, even at low concentrations (Hernández et al., 2007; Pauwels and Verstraete, 2006; Purdom et al., 1994; Schwartz et al., 2003). Nalidixic acid is a synthetic antibacterial agent frequently used in the treatment of urinary tract infections involving Gram-negative organisms (Othman et al., 1988).

Alternatives to the conventional activated sludge treatment are employed for nonbiodegradable or toxic industrial wastewater. Among these, chemical oxidative treatments, and especially, Advanced Oxidation Processes (AOP), are well known for their capacity for oxidizing and mineralizing almost any organic contaminant (Comninellis et al, 2008). Nevertheless, technical applications are still scarce. As the process costs may be considered the main obstacle to their commercial application, several promising cost-cutting approaches have been proposed, such as integration of AOPs as part of a treatment train. In the typical basic process design approach an AOP pretreats nonbiodegradable or toxic wastewater, and once biodegradability has been achieved, the effluent is transferred to a cheaper biological treatment. The key is to minimize residence time and reagent consumption in the more expensive AOP stage by applying an optimized coupling strategy (Scott and Ollis, 1997). Other proposed cost-cutting measures are the use of renewable energy sources, i.e., sunlight as the irradiation source for running the AOP. Photo-Fenton has been successfully demonstrated in real wastewater containing high organic loads in complicated matrixes as a suitable treatment for this purpose (Da Hora Machado et al., 2004; Gernjak et al., 2007; Maciel et al., 2004; Moraes et al., 2004; Rodriguez de Souza et al., 2006).

Nevertheless, there are very few studies that combine the information of chemical analysis, toxicity analysis and biodegradability analysis to study the viability of the combination of photo-Fenton and biological treatment on actual industrial wastewater, not only model wastewater. Some of the few available studies were conducted in our group (Malato et al., 2007; Zapata et al., 2008), but these show different results regarding coupling strategy for different wastewaters. Hence,

Nevertheless, there is still a major need for a scientific rationale on which an "a priori" choice of the most appropriate treatment can be based and additional case-studies like the present one are required to enhance the common knowledge database.

~~Industrial wastewater is often polluted by toxic or nonbiodegradable organic compounds. Special attention currently focuses on pharmaceuticals [Joss et al., 2005; 2006]. Their common consumption in human and veterinary medicine generates a diverse range of residual pollutants (pharmaceuticals + metabolites) that reach the aquatic environment through wastewater [Jones, 2001; Heberer., 2002]. Antibiotics are of particular concern, as they can induce bacterial resistance, even at low concentrations [Hernández et al., 2007; Pauwels and Verstraete, 2006; Purdom et al., 1994; Schwartz et al., 2003]. Nalidixic acid is a synthetic antibacterial agent frequently used in the treatment of urinary tract infections involving Gram-negative organisms [Othman et al., 1988].~~

The aim of this study is to provide a strategy for determining the best way of combining Advanced Oxidation Processes (in this case photo-Fenton) and biological treatment (immobilized biomass reactor) to achieve the mineralization and detoxification of a real pharmaceutical wastewater containing nalidixic acid.

2. Experimental

2.1 Chemicals

The nalidixic acid standard was provided by Fluka (ref. code 70162, 25g). HPLC grade methanol was supplied by Merck (Germany). A Milli-Q ultra-pure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain the HPLC-grade water used in the analyses. Formic acid (purity, 98%) was obtained from Fluka (Germany). Distilled water used in the pilot plant was supplied by the Plataforma Solar de Almería (PSA) distillation plant (conductivity<10 µS/cm, Cl^- = 0.2-0.3 mg/L, NO_3^- <0.2 mg/L, organic carbon <0.5 mg/L). The experiments were performed using iron sulfate heptahydrate ($FeSO_4.7H_2O$), reagent-grade hydrogen peroxide (30% w/v) and sulfuric acid for pH adjustment, all purchased from Panreac. The photo-treated solutions were neutralized by NaOH (reagent-grade, Panreac) for toxicity and biodegradability analyses, and for discharge of the photo-treated sample into the bioreactor. Industrial pharmaceutical wastewater is described in detail in the Results and Discussion section.

2.2 Solar photochemical treatment

All solar photochemical experiments were performed in a pilot-plant made up of Compound Parabolic Collectors (CPCs) designed for solar photocatalytic applications. This reactor is composed of two modules with twelve Pyrex glass tubes mounted on a fixed platform tilted 37º (local latitude). The total illuminated area is 3 m^2 and the volume is 40 liters, 22 l of which are irradiated volume.

At the beginning of all the photo-Fenton experiments, the solutions studied were directly added to the photoreactor, and a sample was taken after 15 min of homogenization (initial concentration). Then the pH was adjusted with sulfuric acid and another sample was taken after 15 min to confirm the pH. Afterwards, iron salt was also added ($FeSO_4.7H_2O$) and homogenized well for 15 min before a sample was taken. Finally an initial dose of hydrogen peroxide was added and samples were taken to evaluate the degradation process.

Photo-Fenton experiments were carried out at a pH adjusted to 2.6–2.8 (H_2SO_4, 2N) and Fe^{2+} concentration of 20 mg/L. In the kinetic study, the initial hydrogen peroxide concentration was around 300 mg/L and was maintained between 200-400 mg/L during the experiments.

Solar ultraviolet radiation (UV) was measured by a global UV radiometer (KIPP&ZONEN, model CUV 3) mounted on a platform tilted 37º (the same as the CPCs). With Equation 1, combination of the data from several days' experiments and their comparison with other photocatalytic experiments is possible (Malato et al. 2003).

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

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), V_i is the total irradiated volume (22.0 L).

2.3 Analytical determinations

The nalidixic acid was analyzed by liquid chromatography (flow rate 0.5 mL/min) in a HPLC–UV (Agilent Technologies, series 1100) with a C-18 column (LUNA 5 μm, 3x150mm, from Phenomenex). The isocratic method used formic acid 25 mM/methanol 50/50, λ=254nm. Ammonium and Na^+ concentration were determined with a Dionex DX-120 ion chromatograph equipped with a Dionex Ionpac CS12A

156 4 mm x 250 mm column. Isocratic elution was done with H_2SO_4 (10 mM) at a flow rate
157 of 1.2 mL/min. Anion concentrations (NO_3^- and Cl^-) were determined with a Dionex
158 DX-600 ion chromatograph using a Dionex Ionpac AS11-HC 4 mm x 250 mm column.
159 The gradient program was pre-run for 5 min with 20 mMNaOH, an 8-min injection of
160 20 mM of NaOH, and 7 min with 35 mM of NaOH, at a flow rate of 1.5 mL/min.
161 Mineralization was followed by measuring the dissolved organic carbon (DOC) by
162 direct injection of filtered samples into a Shimadzu-5050A TOC analyzer with an NDIR
163 detector and calibrated with standard solutions of potassium phthalate. Chemical
164 oxygen demand (COD) was measured with Merck® Spectroquant kits. Total iron
165 concentration was monitored by colorimetric determination with 1,10-phenanthroline,
166 following ISO 6332, using a Unicam-2 spectrophotometer. Hydrogen peroxide was
167 analyzed by a fast, simple spectrophotometric method using ammonium metavanadate,
168 which allows the H_2O_2 concentration to be determined immediately based on a red-
169 orange peroxovanadium cation formed during the reaction of H_2O_2 with metavanadate,
170 maximum absorption of which is at 450 nm. The peroxide concentrations are calculated
171 from absorption measurements by a ratio found by Nogueira et al. (2005).
172
173 **2.4 Toxicity and biodegradability assays**
174 Toxicity of the initial wastewater and selected pre-treated samples was evaluated
175 with Biofix®Lumi-10, a commercial assay. The test is based on the inhibition of the
176 luminescence emitted by the marine bacteria *Vibrio fischeri*. The reagent is a freeze-
177 dried preparation of a specially selected strain of the marine bacterium *Vibrio fischeri*
178 (formerly known as *Photobacterum phosphoreum*, NRRL number B-11177). The drop
179 in light emission was measured after contact periods of 30 minutes. Hydrogen peroxide
180 present in the samples from photo-Fenton experiments was removed prior to toxicity
181 analysis using catalase (2500 U/mg bovine liver; 100 mg/L) acquired from Fluka
182 Chemie AG (Buchs, Switzerland) after adjusting the sample pH to 7.
183 Biodegradability of the photo-Fenton pre-treated pharmaceutical wastewater at
184 different stages was evaluated by a Zahn-Wellens test (an adaptation of the EC protocol,
185 Directive 88/303/EEC). Activated sludge from the El Ejido wastewater treatment plant
186 (Almería-Spain), mineral nutrients and the test material as the sole carbon source were
187 placed together in a 0.25-L glass vessel equipped with an agitator. The test was
188 continued for 28 days at 20-25ºC and under diffuse illumination (or in a dark room).
189 Degradation was monitored by DOC determination of the filtered solution, daily or at

other appropriate regular time intervals. The ratio of DOC eliminated after each interval to initial DOC is expressed as the percentage of biodegradation. Samples analyzed are considered biodegradable when the biodegradation percentage is over 70% (EPA, 1996).

2.5 Biological system

The selected biological reactor is an IBR (Immobilized Biomass Reactor). The IBR consists of a 160-L flat-bottom tank filled with 90-95 L of polypropylene 15-mm Pall Ring supports colonized by activated sludge from the conventional aerobic wastewater treatment plant at El Ejido (Almería). The system is also provided with a 100-L conditioner tank with pH control connected to the IBR through a recirculation pump. The operation flux is 500 L/h. Dissolved oxygen, pH and temperature were automatically measured and registered. Total volume of both tanks was 150 L.

Startup and adaptation of the biological reactor began with the immobilization of the sludge on the ring supports, which took two days. After this, the system was maintained with controlled additions 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). During the whole adaptation process the analytical controls used to evaluate the IBR state were the total suspended solid, DOC, pH and Nitrogen concentration (as ammonium and nitrate).

3. Results and discussion

3.1 Matrix characterization

Firstly, the main parameters of the industrial pharmaceutical wastewater were evaluated (Table 1). One relevant point was the high conductivity, associated with the presence of large amounts of inorganic ions, such as chloride and sodium, found in grams per litre. The sample further contained a significant concentration of suspended solids, a DOC of around 775~~755~~ mg/L and COD (chemical oxygen demand) of 3420 mg/L. The most important organic compound studied in the matrix was nalidixic acid (Fig. 1). The concentration of this compound in the wastewater was 45 mg/L, with the main DOC component being acetate.

An initial biodegradability test was performed at two different dilutions (1:2 and 1:8) of the original wastewater. The 1:2 dilution was selected according to the initial

DOC recommended in Zahn-Wellens standard methodology. The other dilution (1:8) was studied in order to understand the behavior of sludge in contact with small concentrations of nalidixic acid. As shown in Fig 2, both samples were biodegradable.

However, the concentration of nalidixic acid remained constant in both cases. Adaptation also took much longer (around 15 days) for the 1:2-diluted sample, mainly due to the higher nalidixic acid concentration. This pharmaceutical may therefore be considered recalcitrant and possibly inhibitory to sludge metabolism, though not very acutely toxic. Nalidixic acid inhibition of sludge metabolism was also confirmed during photo-Fenton tests, as discussed below.

3.2 Solar photo-Fenton treatment

The first study was the photo-Fenton degradation of 30 mg/L of nalidixic acid standard in dematerialised saline water (5 g/L of NaCl). These conditions were selected to simulate the ionic strength of the real wastewater. The pH was kept at 2.6-2.8, the temperature 30-40 ºC and Fe^{2+} concentration was 20 mg/L that is considered as optimum iron concentration for solar photoreactors selected for this study (Malato et al., 2004). The initial nalidixic acid standard mineralization rate in distilled water and with 5 g/L NaCl was 0.61 mg L^{-1} min^{-1} and 0.38 mg L^{-1} min^{-1}, respectively. The consumption of hydrogen peroxide at 60% mineralisation was 12 mM and 15 mM, respectively. The presence of inorganic species, like Cl^-, affects the photo-Fenton process, as Cl^- acts as a hydroxyl radical scavenger. De Laat and Le (2006) explain this by considering that 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 the photo-Fenton efficiency is also related to the formation of iron(III)-chlorocomplexes, concomitantly with the inhibited formation of iron(III)-hydroperoxide complexes, the reactive species in the photo-Fenton process. Nevertheless, $Cl^\bullet$ and $Cl_2^{\bullet-}$ are strong oxidants (E^o_{SHE}, $Cl^\bullet/Cl^-$= 2.41 V; E^o_{SHE}, $Cl_2^{\bullet-}/2Cl^-$ = 2.09 V) and could also oxidize organic solutes, so that the mineralization rate would not be lowered drastically. Similarly, chloride ions also make higher consumption of hydrogen peroxide necessary for the same mineralization through by Reactions 5-6. De

Laat and Le (2006) conclude that over 100 mM are needed to inhibit formation of iron(III)-hydroperoxide complexes and reduce the reaction rate, and that $Cl^{\bullet}$ and $Cl_2^{\bullet-}$ are reactive enough to degrade a wide range of organic compounds. Therefore, the mineralization rate of nalidixic acid dissolved in saline water (5 g/L NaCl) could be expected not to be drastically lowered.

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

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

Figure 3 shows photo-Fenton treatment of the real wastewater described in Table 1. The experimental conditions were 20 mg/L of total iron and H_2O_2 concentration was maintained between 200 and 400 mg/L. 90% of the initial DOC was removed in 400 minutes of illumination time and the total H_2O_2 consumed was 180 mM. Nalidixic acid had completely disappeared at 190 minutes with 72 mM of H_2O_2 consumed. In view of these considerations, it is not recommended to mineralize 90% of DOC, because of the very long time and huge amount of hydrogen peroxide needed (around 6 g/L) for such a high mineralization level. Moreover, the results shown in Figure 2 show that the biodegradability of the wastewater was not bad, and therefore, nalidixic acid degradation is the main goal of treatment. So it is important to determine the behavior of biodegradability and toxicity of wastewater during the photo-Fenton treatment, mainly during nalidixic degradation and shortly afterwards to find the best treatment time with the minimum hydrogen peroxide consumption.

3.3 Toxicity and biodegradability assays

Toxicity and biodegradability of the real wastewater treated by photo-Fenton were evaluated at different stages of the process in order to determine the optimal point for coupling to the biological process.

To do this, it was necessary to reproduce the previous experiment maintaining all the parameters except H_2O_2 dosing the same. H_2O_2 was added so samples would be representative of the photocatalytic process. H_2O_2 was added (a small quantity, on the order of a few mM) to the photoreactor, and consumption monitored, and after the peroxide was consumed, a sample was taken for bioassay. H_2O_2 was again added, and another sample was taken after all the peroxide had been consumed. This procedure of "addition-total consumption-addition" was repeated until significant mineralization

(70%), ensuring that no H_2O_2 remained (which could affect the bioassays). It also prevents any reaction in the dark after the sample is taken form the photoreactor, since analyses are not performed until the H_2O_2 has been completely consumed. Moreover, as nalidixic acid and DOC are also determined in these experiments, results can be compared with the kinetic results shown in Figure 3.Another option could be to work with excess H_2O_2 (as the experiment shown in Figure 3), and eliminate the remaining H_2O_2 before applying the bioassay. But methods for eliminating H_2O_2 based on catalase, MnO_2, etc, could change the matrix composition and/or the added chemicals could change the response of the bioassays. Therefore, the data shown are directly related to H_2O_2 consumption, instead of illumination time. In this experiment, 12 samples suitable for analyzing toxicity and biodegradability were taken (Figure 4).

Total degradation of nalidixic acid was attained at 66 mM of H_2O_2 consumed, a consumption very similar to the experiment shown in Figure 3, confirming that experiments performed by the "addition-total consumption-addition" procedure are comparable to others in which excess H_2O_2 is added (a certain amount of H_2O_2 produces a specific DOC mineralization and nalidixic acid degradation). A first group of samples was selected for the *Vibrio fischeri* toxicity bioassay and COD analysis (S_1, S_5, S_6, S_7, S_8, S_9, S_{11}, S_{12}, S_{13}, S_{14}, S_{15}), and a second group for *Daphnia magna* toxicity analysis and Zahn-Wellens test (S_6, S_9, S_{10}, S_{12}, S_{13}, S_{14}).

Vibrio Fischeri toxicity was evaluated at dilution of 1:3, 1:6 and undiluted. Figure 5 shows only results with the 1:3 dilution along with DOC. All the toxicity results (diluted and undiluted) were quite similar, and it can therefore be concluded that photo-Fenton treatment did not decrease *Vibrio Fischeri* toxicity. *Daphnia magna* bioassays demonstrated similar behavior. All microcrustaceans in all samples died in 24 hours. Therefore, toxicity bioassays show that photo-Fenton was unsuccessful. But both bioassays have being described as very sensitive (Hernando et al., 2007) and this result is not surprising for real wastewater containing hundreds of mg/L of different organic compounds (fro example, carboxylic acids) and their degradation intermediates.

Sometimes toxicity tests (usually a quick method) can help select the stage of an AOP treatment at which the water becomes nontoxic and, presumably, biodegradable (Gutiérrez et al., 2002; Hernando M.D. et al., 2005; Lapertot M. and Pulgarin C., 2006; Lapertot M. et al., 2008). In other words, toxicity tests such as *Vibrio fischeri* can detect

toxic response in a short time (5 to 30 minutes), should be retested afterwards for biodegradability (usually a more time-consuming method). But in view of the results shown in Figure 5, no such information was found from the toxicity assays.

Variation in the COD during the experiment was also determined along with DOC (Figure 6). The considerable decrease in this parameter agrees with the strong oxidation of organic matter. The efficiency of the oxidative process is more clearly shown by the AOS parameter (average oxidation state), which can be calculated by Equation 7, in which DOC and COD are expressed in moles of C/L and of O_2/L, respectively, at the sampling time. AOS is between +4 for CO_2, the most oxidized state of C, and -4 for CH_4, the most reduced state of C. As observed in Figure 6, the maximum AOS of -0.3 was reached after approximately 25 mM of H_2O_2 consumed and remained around there until the end of the test. AOS usually increases with treatment time until almost reaching a plateau. These results suggest that more oxidized organic intermediates are formed at the beginning of the photocatalytic process, and after a certain time, the chemical nature of most of them no longer varies substantially (Sarria, et al., 2002), even if the photo-Fenton treatment continues. Formation of more oxidized intermediates indirectly demonstrates that the treatment can improve biodegradability. At the moment that AOS stabilizes, the chemical treatment is only mineralizing organic contaminants, but with no partial oxidation. The changes in AOS were taken into account in selecting the treatment stage at which the water might presumably be biodegradable. Zahn-Wellens tests (usually a time-consuming method) were therefore only done on samples > S_6 (H_2O_2 consumption 20 mM). Before this treatment stage the concentration of nalidixic acid and toxicity was rather high and AOS increased.

$$\mathbf{AOS} = \frac{\mathbf{4(DOC - COD)}}{\mathbf{DOC}} \qquad (7)$$

Zahn-Wellens tests (Figure 7) were performed on six undiluted samples at different stages during the photo-Fenton process. All samples were at least 90% biodegradable at the end of the Z-W assay. In three of them (S7, S10 and S11), in which nalidixic acid concentrations were 20.7, 8.5 and 4.6 mg/L, respectively, 70% biodegradability was attained only after 10 days of biotreatment (Samples S10 and S11 after 8 days). On the other hand, samples with very low concentrations (< 1 mg/L) or without nalidixic acid (S13, S14 and S15) were biodegradable after 3 days. Results show that untreated samples need much longer adaptation periods than treated samples and that nalidixic acid (at concentration as low as 4.6 mg/L) are also detrimental in the

sense that they reduce biodegradation efficiency. These results demonstrated that photo-Fenton should be performed until total degradation of nalidixic acid before coupling a biological treatment and that AOS determination is an appropriate technique for selecting those samples to be tested by Z-W assay.

3.4 Combined solar photo-Fenton and biological system

When the best photo-Fenton wastewater pretreatment for biodegradability had been determined, the combined photo-Fenton/biological treatment was carried out in a pilot bioreactor. Before performing the experiment in the combined system, the IBR was inoculated with 150 L of concentrated activated sludge from the El Ejido wastewater treatment plant (Almería, Spain). Then, recirculation was maintained between the conditioner tank and the IBR in order to ensure optimum fixation of the sludge on the propylene Pall Ring supports. Total suspended solids, DOC and inorganic ion concentration (mainly ammonia and nitrate) were measured daily. The system was maintained with controlled addition of glucose, a ~~biodegradable~~ pharmaceutical (CAS number: 1953-04-4) less readily biodegradable than glucose, and ammonium chloride, keeping the carbon/nitrogen ratio at 100/20. The next step was adapting the sludge to high salinity, as the wastewater contains large quantities of NaCl (see Table 1). During the whole adaptation process, NaCl concentration was increased gradually in five cycles from 1 to 5 g/L.

Startup and adaptation of the biological reactor was done while simultaneously performing several runs with the photo-Fenton reactor in order to accumulate enough pre-treated wastewater to add to the bioreactor, as the photo-Fenton plant only has a 40 L volume and the bioreactor (IBR+conditioner tank) has 150 L. The different photo-Fenton runs were accumulated in a large volume tank (neutralization tank) connected to the bioreactor where the pH was adjusted roughly to 7, as automatic pH control is in the bioreactor itself. In the coupled system, wastewater containing nalidixic acid (45 mg/L) was pre-treated by photo-Fenton until its total elimination during 190 minutes of illumination time with 20mg/L of Fe^{2+} and 66 mM of H_2O_2 consumed. The chemical characterization of the photo-Fenton effluent was 530 mg/L of DOC and 6.5 g/L of NaCl. It should be remarked that the same dose of H_2O_2 (66 mM) did not always accomplish exactly the same mineralization, as observed in Figure 4, where less than 530 mg/L were attained. But the scope of the treatment was the complete (< 1 mg/L)

degradation of nalidixic acid. The pre-treated effluent was pumped from the neutralization tank to the conditioner tank connected to the IBR.

The system was operated in batch mode, with a recirculation flow rate of 500 L/h between the conditioner tank and the IBR until the effluent was bio-mineralized (final DOC 35 mg/L). The DOC and evolution of nitrogen (as NH_4^+ and NO_3) during biological treatment are shown in Figure 8. DOC went down 495 mg/L in 4 days, a result similar to the Z-W test. NH_4^+ (NH_4Cl, in a 68 mg/L concentration of N) was added the first day to enable nitrifying bacteria to metabolize the organic carbon. The consumption of $NH_4^+$and the generation of NO_3^- demonstrated nitrification and N assimilation through biomass grow as overall N content decreased.

Overall mineralisation efficiency of the combined photo-Fenton and biological treatment in batch mode for the degradation of the real pharmaceutical wastewater was over 9597%, of which 3327% corresponds to the solar photochemical process and 6270% to the biological treatment. *Vibrio Fischeri* and *Daphnia magna* bioassays were also performed on the biotreatment effluent showing below-threshold toxicity. Therefore, the combined treatment was also successful from the viewpoint of biotoxicity.

4. Conclusions

- It has been demonstrated that a toxic industrial wastewater containing a biorecalcitrant compound (nalidixic acid) can be successfully treated by photo-Fenton after long treatment with heavy consumption of hydrogen peroxide, but without decreasing toxicity.
- Photo-Fenton successfully enhanced the wastewater biodegradability.
- Suitable selection of the photo-Fenton treatment time and hydrogen peroxide dose necessary to reach the biodegradability threshold made it possible to degrade the remaining DOC in a pilot aerobic bioreactor, and detoxify the wastewater.
- The global efficiency in the combined solar photo-Fenton+IBR system operated in batch mode was 9597% of DOC elimination (initial DOC of 775820 mg/L), of which 3327% was accomplished by the solar photo-Fenton treatment and 6270% by the biological treatment.

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517 and Biological Treatment for Detoxification of Pharmaceutical Industry Contaminants.
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519 **Table 1:** Main parameters of industrial pharmaceutical wastewater

520

Parameter	Amount
pH	3.98
Conductivity	7 $mS.cm^{-1}$
DOC	775 $mg.L^{-1}$
COD	3420 $mg.L^{-1}$
Acetate	1.9 $g.L^{-1}$
Nalidixic acid	45 $mg.L^{-1}$
TSS	0.407 $g.L^{-1}$
Cl^-	2.8 $g.L^{-1}$
PO_4^{3-}	0.01 $g.L^{-1}$
SO_4^{2-}	0.16 $g.L^{-1}$
Na^+	2 $g.L^{-1}$
Ca^{2+}	0.02 $g.L^{-1}$

521

522

523 **FIGURE CAPTIONS**

524

525 **Figure 1:** Chemical structure of nalidixic acid.

526

527 **Figure 2:** Zahn-Wellens biodegradability test of the pharmaceutical wastewater: 1:2 and
528 1:8 dilutions.

529

530 **Figure 3:** Mineralization of industrial pharmaceutical wastewater and H_2O_2 consumed
531 during photo-Fenton. Inset shows degradation of nalidixic acid during the same test.

532

533 **Figure 4:** Mineralization of real wastewater during the photo-Fenton process as
534 function of hydrogen peroxide dose (addition-total consumption-addition procedure). S_n
535 (from S_1 to S_{17}) are selected samples for toxicity and biodegradability studies.

536

537 **Figure 5:** Toxicity bioassay (*Vibrio fischeri*) of 1:3 diluted samples and DOC during
538 the photo-Fenton process.

539

540 **Figure 6:** AOS (see Equation 7) evolution during the photo-Fenton process.

541

542 **Figure 7:** Zahn-Wellens test for selected samples during the photo-Fenton process.
543 (Initial sample is also shown)

544

545 **Figure 8:** Mineralisation of the photo-Fenton pre-treated wastewater in the IBR.

546

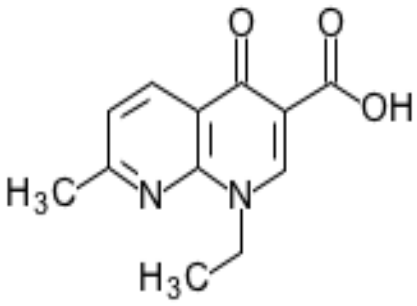

Fig. 1

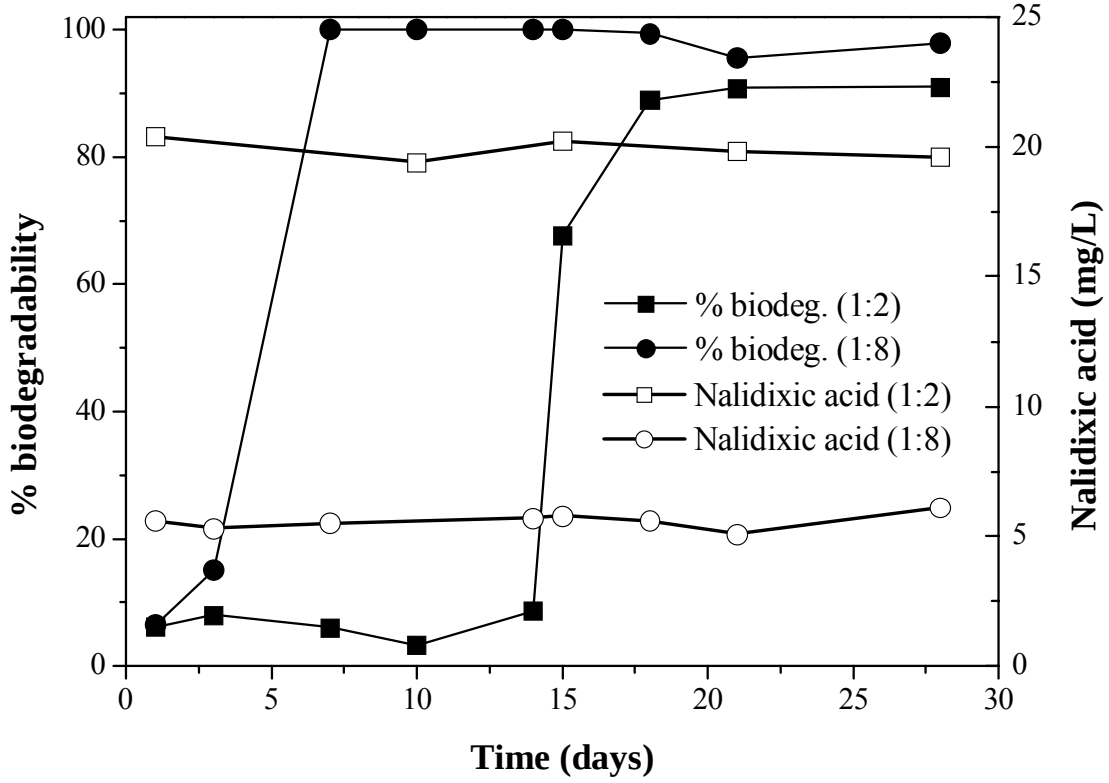
% biodegradability
Nalidixic acid (mg/L)
Time (days)
% biodeg. (1:2)
% biodeg. (1:8)
Nalidixic acid (1:2)
Nalidixic acid (1:8)
0
20
40
60
80
100
0
5
10
15
20
25
30

Fig. 2

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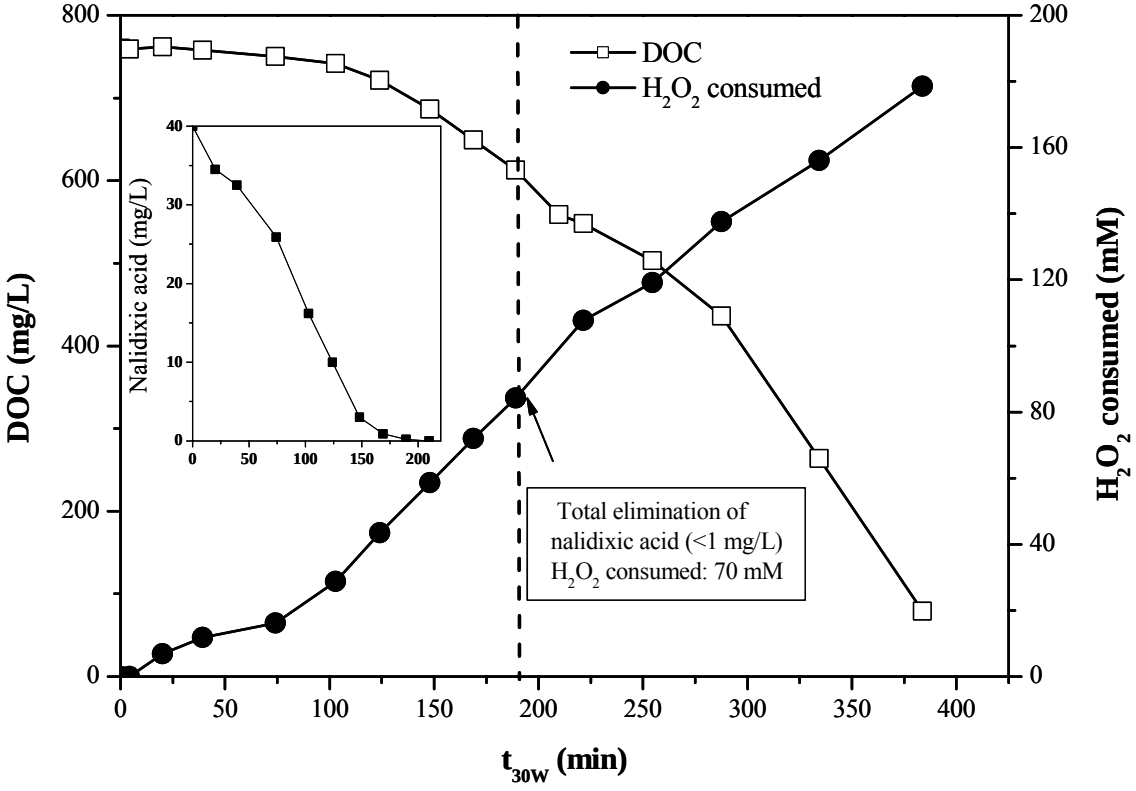

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568 Fig. 3

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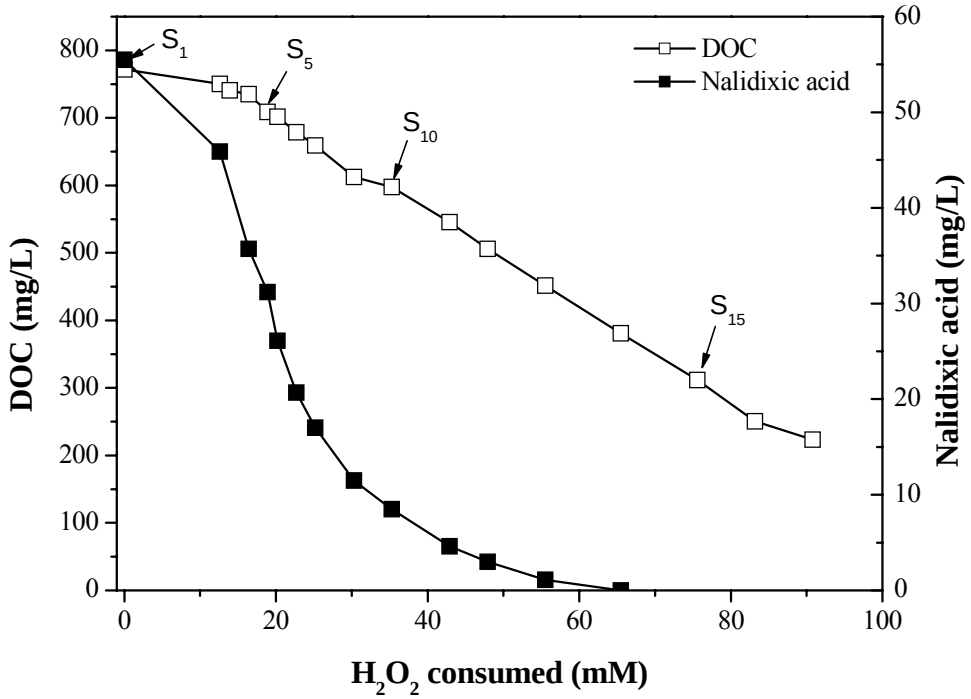

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573
574 Fig. 4
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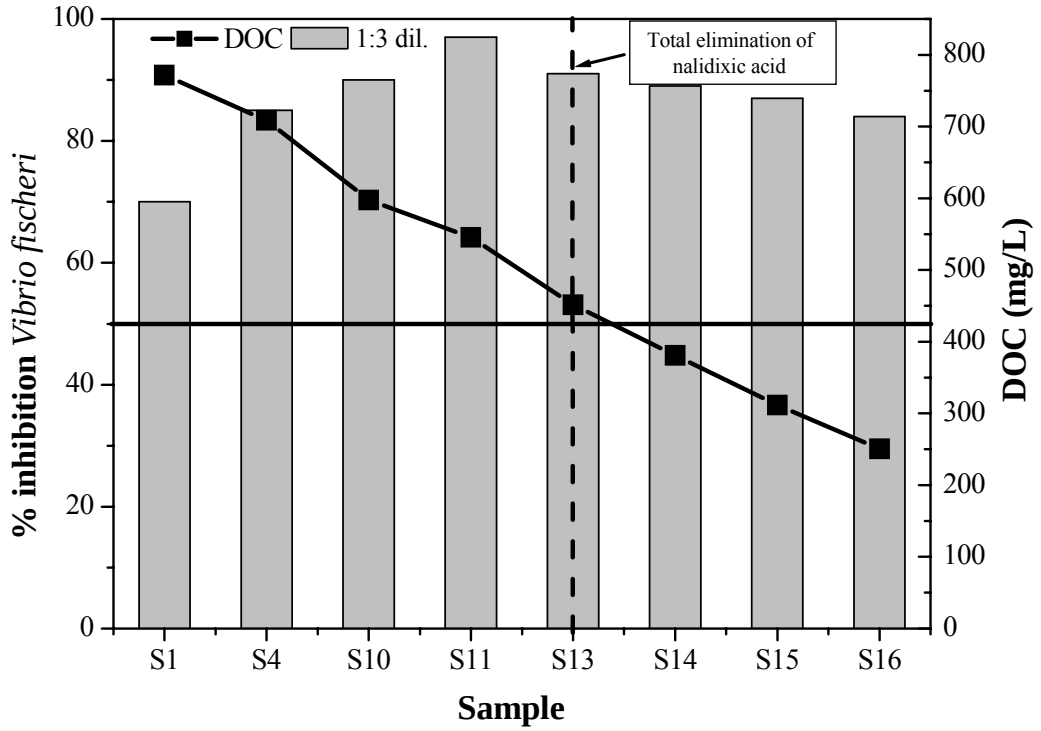

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584 Fig. 5

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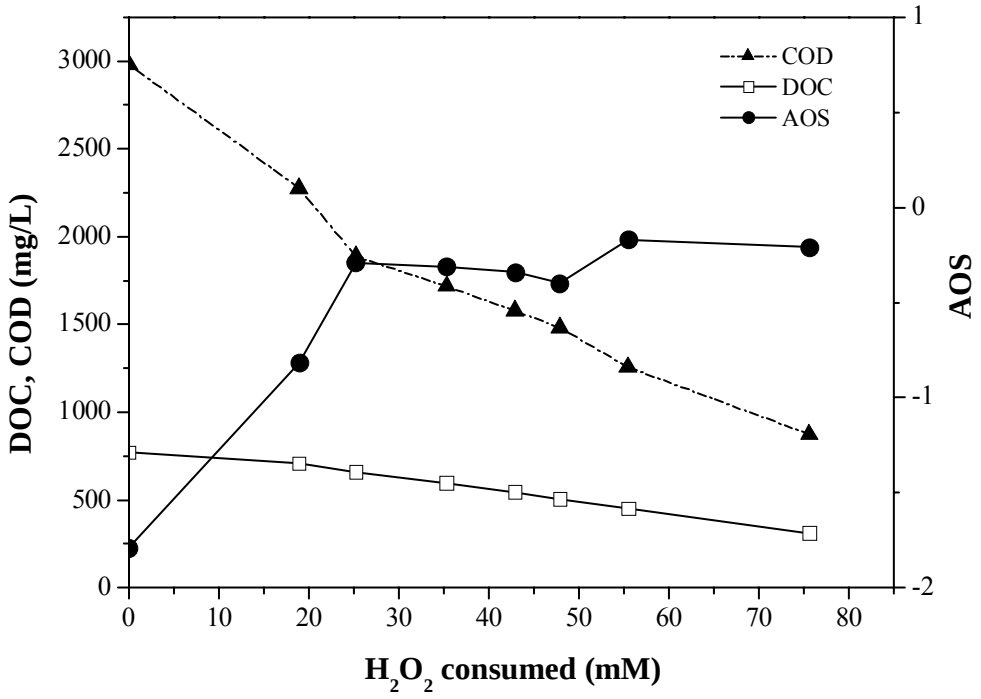

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592 Fig. 6

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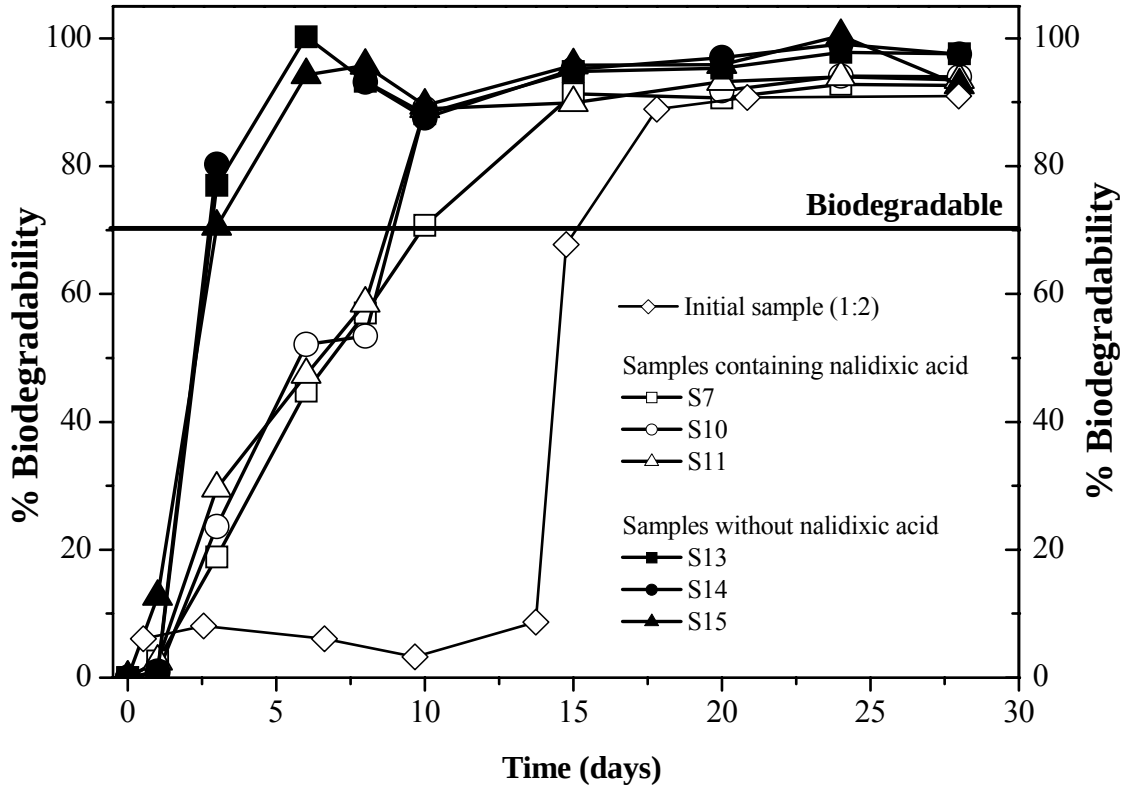
% Biodegradability
Time (days)
Biodegradable
Initial sample (1:2)
Samples containing nalidixic acid
S7
S10
S11
Samples without nalidixic acid
S13
S14
S15
0
20
40
60
80
100
0
5
10
15
20
25
30

Fig. 7

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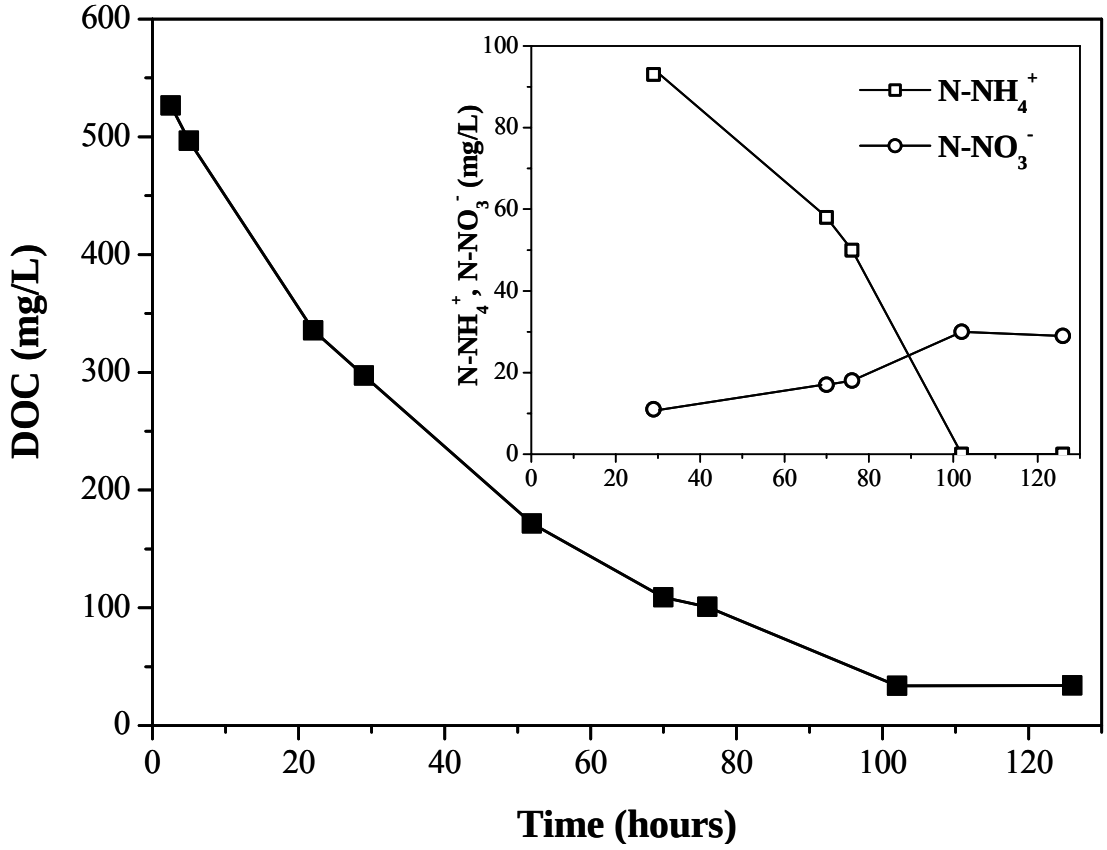

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606 Fig. 8

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