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The testing of several biological and chemical coupled treatments for Cibacron Red FN-R azo dye removal

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Abstract

Several biological and chemical coupled treatments for Cibacron Red FN-R reactive azo dye degradation have been evaluated. Initially, a two-stage anaerobic–aerobic biotreatment has been assessed for different dye concentrations (250, 1250 and 3135 mg l^{-1}). 92–97% decolourisation was attained during the anaerobic digestion operating in batch mode. However, no dissolved organic carbon (DOC) removal neither biogas production was observed during the process, indicating that no methanogenesis occurred. Additionally, according to Biotox® and Zahn–Wellens assays, the anaerobically generated colourless solutions (presumably containing the resulting aromatic amines from azo bond cleavage) were found to be more toxic than the initial dye as well as aerobically non-biodegradable, thus impeding the anaerobic–aerobic biological treatment. In a second part, the use of an advanced oxidation process (AOP) like photo-Fenton or ozonation as a chemical post-treatments of the anaerobic process has been considered for the complete dye by-products mineralisation. The best results were obtained by means of ozonation at pH 10.5, achieving a global 83% mineralisation and giving place to a final harmless effluent. On the contrary, the tested photo-Fenton conditions were not efficient enough to complete oxidation.

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Keywords: Aerobic biodegradation; Anaerobic digestion; AOPs post-treatment; Reactive azo dye

1. Introduction

Azo dyes are the most widely used class of industrial dyes, constituting 60–70% of all produced dyestuffs [1]. During the dyeing process, the degree of exhaustion of dyes is never complete resulting in dye-containing effluents [2]. Not only aesthetic problems occur, but also biotoxicity and the possible mutagenic and carcinogenic effects of azo dyes or their metabolites have been reported [3]. Their degradation by conventional wastewater treatment technologies is difficult due to their complex structure and synthetic nature; they are non-biodegradable by standard aerobic activated sludge methods, and systems based on physical and chemical methods are quite inefficient and require further treatment or disposal of the pollutant. Therefore, the develop-

ment of effective and economic methods for textile wastewaters treatment should deserve particular attention. Not only colour removal but organic degradation of dyes and their intermediates must also be the pursued goals.

Among the emerging technologies for wastewater decontamination, advanced oxidation processes (AOPs) are capable to mineralise almost all toxic and non-biodegradable organic compounds [4]. These processes are principally based on the in situ generation of highly reactive hydroxyl radicals (HO•, $E° = 2.8$ V versus NHE). The most widely studied AOPs include: heterogeneous photocatalytic oxidation with TiO_2 [5,6], treatment with ozone (often combined with H_2O_2 and/or UV) [7,8], UV/H_2O_2 systems [9], and Fenton and photo-Fenton reactions [10–12], which generate HO• by interaction of H_2O_2 with a ferrous salt in aqueous media. Nevertheless, the operational costs associated are a common problem of all AOPs and they often are too expensive to be applied as exclusive treatment. In this sense, several earlier studies focus on the combination of an AOP with

conventional aerobic biological treatment in an attempt to avoid the drawbacks of each of them. A minimum oxidation is performed as a pre-treatment to just increase the biodegradability and generate a new effluent more amenable to biodegradation [13–15].

On the other hand, since azo dyes are potentially reduced under anaerobic conditions, and biological processes are the preferred choice for wastewater treatment, biotreatments based on anaerobic–aerobic sequences are currently under investigation [16–18]. Azo bonds ($R_1-N=N-R_2$) can be anaerobically reduced basically leading to the corresponding aromatic amines (dye solution decolourisation), compounds that are recalcitrant and toxic under reducing atmosphere but theoretically susceptible to further aerobic biodegradation [19].

In a previous work, the coupling of photo-Fenton reaction and an aerobic biological process for the degradation of a representative reactive azo dye employed for cellulosic fibres dyeing ($250 \, mg \, l^{-1}$ hydrolysed Cibacron Red FN-R, DOC = $80 \, mg \, l^{-1}$ C) was carried out [15]. At the studied concentration, the dye was neither aerobically biodegradable nor toxic ($EC_{50} > 80 \, mg \, l^{-1}$ C) as seen by Zahn–Wellens and Biotox® assays, respectively. With the application of the photo-Fenton catalytic process as a biological pre-treatment, a complete decolourisation and 80% mineralisation was accomplished in the combined oxidation system.

In this paper, several biological and chemical coupled treatments for Cibacron Red FN-R azo dye removal were evaluated as alternatives to the chemical–biological (aerobic) strategy. In a first part, the possibility of Cibacron Red FN-R anaerobic digestion coupled to an ensuing aerobic degradation was tested. Anaerobic assays were carried out in batch mode. Biogas production, % decolourisation, DOC reduction and toxicity evolution after digestion were monitored in order to evaluate the success of the anaerobic stage. The Zahn–Wellens test was carried out for the aerobic biodegradability assessment of the resulting anaerobic effluent. In a second part, the application of an AOP as a chemical post-treatment of the anaerobic process has also been considered to complete reactive azo dye and by-products mineralisation. Ozonation and photo-Fenton processes have been chosen to play this role.

2. Materials and methods

2.1. Synthetic dye solution

A commercial reactive azo dye, Cibacron Red FN-R bireactive vinylsulphone fluorotriazine dye (CI Reactive Red 238, empirical formula $C_{29}H_{15}O_{13}S_4ClFN_7Na_4$, $944.2 \, g \, mol^{-1}$) was purchased from CIBA and used as received without further purification (80% purity approx.). Its chemical structure was not disclosed by the manufacturer. The different Cibacron Red FN-R concentrations were prepared by diluting an initial concentrated stock solution of $4180 \, mg \, l^{-1}$ (DOC = $1213 \, mg \, l^{-1}$ C; chemical oxygen demand (COD) = $3433 \, mg \, l^{-1}$ O_2). The initial solution of Cibacron Red FN-R was hydrolysed to convert it to the form in which it is normally found in industrial effluents. The hydrolysis was done by adjusting the pH to 10.6, followed

by heating to 60 °C for 1 h. Finally, the pH of the hydrolysed stock solution was adjusted to 7.0 ± 0.2 before storage at 4 °C.

2.2. Anaerobic biodegradation set-up and operation conditions

The anaerobic experiments were conducted in batch mode and under static conditions in 600 ml thermostated aluminium bottles. The test sample volume was 500 ml and the headspace was 100 ml. The anaerobic sludge seed was a methanogenic culture obtained from a mesophilic municipal anaerobic digester (Manresa, Spain), containing an initial total suspended solids (TSS) and a volatile suspended solids (VSS) of 30 and $12 \, g \, l^{-1}$, respectively. All bottles were seeded with the same volume of original sludge to finally obtain an initial $1.5 \, g \, l^{-1}$ VSS and $3.8 \, g \, l^{-1}$ TSS concentration.

0.2 g of yeast extract and 2 ml of the following solutions were added per litter of sample [20]: $100 \, mg \, l^{-1}$ $Na_2S \cdot 9H_2O$ solution; a macronutrients solution composed of $170 \, g \, l^{-1}$ NH_4Cl, $37 \, g \, l^{-1}$ KH_2PO_4, $8 \, g \, l^{-1}$ $CaCl_2 \cdot 2H_2O$ and $9 \, g \, l^{-1}$ $MgSO_4 \cdot 4H_2O$; a micronutrients solution composed of $2000 \, mg \, l^{-1}$ $FeCl_3 \cdot 4H_2O$, $2000 \, mg \, l^{-1}$ $CoCl_2 \cdot 6H_2O$, $500 \, mg \, l^{-1}$ $MnCl_2 \cdot 4H_2O$, $30 \, mg \, l^{-1}$ $CuCl_2 \cdot 2H_2O$, $50 \, mg \, l^{-1}$ $ZnCl_2$, $50 \, mg \, l^{-1}$ H_3BO_3, $90 \, mg \, l^{-1}$ $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, $100 \, mg \, l^{-1}$ $Na_2SeO_3 \cdot 5H_2O$, $50 \, mg \, l^{-1}$ $NiCl_2 \cdot 6H_2O$, $1000 \, mg \, l^{-1}$ EDTA, $1 \, ml \, l^{-1}$ HCl 36% and $500 \, mg \, l^{-1}$ resazurin. Additionally, 1 g of $NaHCO_3$ per g COD was added to the solution in order to maintain the pH and reduce the negative effects to methanogenesis due to the possible acidification caused by volatile fatty acids (VFA) generation. Before incubation, nitrogen gas was bubbled into the bottles to ensure anaerobic conditions during the biodegradation process. Temperature was maintained under mesophilic conditions at 37 ± 1 °C. Each experiment was extended to 50 days.

Biogas production was measured at regular intervals by monitoring pressure changes in the bottles headspace with a digital manometer (SMC Pressure Switch 10 bars) connected to the bottles by a valve. The methane composition was determined using a HP 5890 gas chromatograph equipped with a thermal conductivity detector and a Porapak Q, 3 m × 1/8 in. column (Supelco). The temperature of the injector was 130 °C. The oven temperature was initially maintained at 30 °C for 3 min. Then, it was increased to 70 °C at a 10 °C min^{-1} rate and finally maintained at 70 °C for 5 min. The temperature of the detector was 180 °C.

The methanogenic activity of the anaerobic sludge was ensured by the assessment of the specific methanogenic activity (SMA) of a VFA standard solution composed of acetic, propionic and butyric acids (73:21:4 weight proportion, total COD = $4500 \, mg \, l^{-1}$ O_2) [20]. From the maximum biogas generation slope (60–70% in CH_4), a SMA value of $0.084 \, gCOD_{CH_4} \, g^{-1}VSS \, day^{-1}$ was obtained (typical SMA values of anaerobic biomass from a municipal anaerobic digester range between 0.02 and $0.2 \, gCOD_{CH_4} \, g^{-1}VSS \, day^{-1}$ [20]).

To analyse dye solutions, samples were centrifuged and the supernatant was filtered through $0.45 \, \mu m$ pore size filters. Prior toxicity testing, samples were gassed with nitrogen to strip out

any possible remaining H_2S. Every assay was performed at least twice.

2.3. Adsorption assay

Adsorption experiments were conducted in 100 ml closed thermostated flasks at $37 \pm 1\,^\circ C$ containing $1.5\,g\,l^{-1}$ VSS and $3.8\,g\,l^{-1}$ TSS of anaerobic sludge and 30 ml of dye solutions with 85, 250, 425, 850, 1275, 1700, 2550 and $3000\,mg\,l^{-1}$ concentrations. Neither nutrients nor trace elements were added into the media. The contact time was set at 24 h and pH 7.0 ± 0.2. One flask with biomass suspended in deionised water was used as control. Hand shaking was occasionally conducted. Duplicates were carried out for every concentration.

2.4. Photo-Fenton and ozonation set-up

Photo-Fenton and ozonation processes were conducted in a thermostated ($T = 23 \pm 1\,^\circ C$) well stirred cylindrical Pyrex cell of 300 ml of capacity. The solution volume was 200 ml. In photo-Fenton process, analytical grade hydrogen peroxide (33%, w/v, Panreac) and $FeSO_4 \cdot 7H_2O$ (99.5%, Merck) were used as received to generate hydroxyl radical ($HO^\bullet$) in aqueous solution. A 6 W Philips black light fluorescent lamp placed over the cell was used as artificial light source. The intensity of the incident UVA light, measured employing a luminometer, was $0.6\,mW\,cm^{-2}$. The pH of the solutions was adjusted to 2.8–3.0. Ozone was generated by an Erwin Sander 301.7 model equipment fed with a pure oxygen stream. To ensure saturation of the system the input in the solution was $1.75\,g\,h^{-1}$ O_3 as determined by iodometric titration [21]. Unreacted ozone was measured by means of an Erwin Sander Quantozone-1 ozonemeter and finally destroyed with a KI trap. Prior toxicity testing, solutions were gassed with nitrogen to strip out the remaining dissolved O_3 gas.

2.5. Chemical analysis

UV/vis-absorption spectra were recorded by using a Shimadzu UV-1603 double beam spectrophotometer in the 200–700 nm range. All colour data were reported as the absorbance at the maximum absorption in the visible region ($\lambda_{max} = 542.5$ nm), and were taken as an estimation of the dye presence in solution. Samples were diluted prior to measurements if necessary. DOC was determined with a Shimadzu TOC-V$_{CSH}$ analyser. COD was assessed by the closed reflux colorimetric method [22] with a HACH DR/2000 spectrophotometer. Determination of TSS and VSS was carried out following Standard Methods [22].

2.6. Biological assays

Non-acclimated activated sludge coming from a wastewater treatment plant (Manresa, Spain) was used to perform the Zahn–Wellens test [23]. Acute toxicity was assessed using the Biotox® bacterial bioluminescence assay [24], by determination of the inhibitory effect that the sample has on the marine pho-

tobacteria *Vibrio fischeri*. The toxicity effect was quantified as the EC_{50} parameter (the concentration of toxicant that causes 50% decrease of light emission after 30 min of exposure) and was expressed in DOC units (mg l^{-1} C). When the DOC of the sample was lower than the EC_{50}, this was assumed to be non-toxic and toxicity values were given as $EC_{50} > 100\%$. Phenol and glucose solutions were used as standard toxic and non-toxic samples to check the technique suitability. Colour correction was applied in toxicity determinations of coloured solutions.

3. Results and discussion

3.1. Adsorption study

Since biological dye removal from wastewaters may involve both adsorption and biodegradation by bacteria [17], an adsorption test was conducted in order to investigate the potential of Cibacron Red FN-R adsorption on the anaerobic sludge. DOC and colour (Abs$_{542.5}$) parameters were used as indicators. After 24 h of contact time, neither significant DOC nor colour reduction was observed for any concentration tested, discarding a possible biosorption mechanism for colour and organic removal during anaerobic digestion. The repulsive electrostatic interaction between Cibacron Red FN-R reactive azo dye (of anionic nature) and the negative charges of anaerobic sludge might be responsible for the lack of biosorption observed [25].

3.2. Cibacron Red FN-R anaerobic digestion

Three different Cibacron Red FN-R concentrations were tested in order to assess the anaerobic sludge response to azo dye dosage: 250, 1250 and $3135\,mg\,l^{-1}$ (bottles 1, 2 and 3). High dye concentrations were employed to ease the quantification of the potentially formed biogas. On the other hand, to obtain information about Cibacron Red FN-R anaerobic degradation in presence of an auxiliary substrate – which may be required for generation of reducing equivalents consumed during azo bond cleavage [19] –, $2500\,mg\,l^{-1}$ of yeast extract was added to another $1250\,mg\,l^{-1}$ dye solution (bottle 4). As a basis for comparison, a control bottle just containing anaerobic biomass with the corresponding nutrients and trace elements (control I), and another also containing $2500\,mg\,l^{-1}$ yeast extract (control II) were run in parallel.

The parameters employed to evaluate the biodegradation efficiency were the maximum methane generation slope per unit of weight of biomass (gCOD$_{CH_4}$ g^{-1}VSS day^{-1}), a magnitude similar to the SMA (although SMA is specifically defined to quantify standards activity) that corresponds to the maximum value of the sample methanogenic activity along one experiment (usually occurs at the beginning of the anaerobic process, after an idle time of biomass adaptation) [20], the accumulated biogas production within the 50 days of assay (1 atm, $37\,^\circ C$), as well as the % decolourisation (Abs$_{542.5}$ determination) and DOC reduction after the 50 days of experiment (Table 1).

From Table 1, it is noteworthy that nearly complete decolourisation had taken place after digestion of all substrate types (even when no additional yeast extract was present). 92–97% of the

Table 1
Summary of experimental results for different Cibacron Red FN-R and control solutions after 50 days of anaerobic digestion

Bottle	Substrate type	% Decolourisation (Abs$_{542.5}$)	DOC mg l^{-1}C			gCOD$_{CH_4}$ g^{-1} VSS day^{-1}	Accumulated biogas ratio
			Initial	Residual	Removed		
Control I	–	–	105[a]	29	76	0.005	1
1	250 mg l^{-1} dye	92	176	96	80	0.004	0.89
2	1250 mg l^{-1} dye	96	467	416	51	0.005	0.49
3	3135 mg l^{-1} dye	97	1013	979	34	0.005	0.38
Control II	2500 mg l^{-1} yeast extract	–	1107	43	1064	0.042	1
4	2500 mg l^{-1} yeast extract, 1250 mg l^{-1} dye	93	1471	609	862	0.038	0.65

[a] DOC corresponding to organic nutrients supplied to all the bottles.

visible band at 542.5 nm, characteristic of azo bonds and responsible for the red colour of the solution [26], disappeared within the anaerobic treatment. However, DOC data evidence that no methanogenesis from dye by-products had taken place during the anaerobic stage. When comparing the control experiments with the dye-containing experiments (the DOC content of control I is due to biodegradable organic nutrients supplied to all the bottles (see Section 2.2)), none of the dye concentrations showed higher DOC removal that the attained by the control experiments. DOC reduction was almost the same for the 250 mg l^{-1} Cibacron Red FN-R solution and control I experiment, and even lower for higher dye dosages (Table 1), evidencing a possible toxic/inhibitory effect to anaerobic biomass. The DOC removal of 1250 mg l^{-1} dye plus 2500 mg l^{-1} yeast extract sample was also lower than control II experiment.

Biogas measurements show similar results. Regarding the maximum methane generation slope, it is noteworthy that none of the dye samples provided larger values than the corresponding to control I and II activities, which showed 0.005 and 0.042 gCOD$_{CH_4}$ g^{-1}VSS day^{-1} values, respectively. On the other hand, when comparing the accumulated biogas production of dye solutions with the accumulated biogas production of control bottles for a period of 50 days (data expressed as accumulated biogas ratio in Table 1), they were never higher than control (ratio values lower than unity) and they decreased when dye concentration was increased. From these results, a possible toxic/inhibitory effect of solutions on anaerobic biomass is also deducible. As an example, Fig. 1 shows the accumulated biogas evolution obtained in several of the experimental bottles. At first, it is noticeable a lag phase with no biogas production probably due to inoculum adaptation to both wastewater and operational conditions. Subsequently, the maximum anaerobic degradation takes place, with a continuous 60–70% methane generation (at this point maximum methane generation slope values were obtained). Finally, when compared with control experiments, total biogas and methane production of dye-containing bottles slowed down. This phenomenon is clearly observed with the 1250 mg l^{-1} dye plus 2500 mg l^{-1} yeast extract sample.

Some conclusions can be extracted from the above results. In absence of a dye biosorption mechanism (see Section 3.1), and in agreement with the bibliography previously reported, Cibacron Red FN-R reactive azo dye decolourisation would be attributable to the reductive cleavage of azo bonds (dye chromophore) giving place to the resultant biorecalcitrant aromatic amines. Their biorecalcitrant nature would induce the negligible dye DOC removal and the lack of methanogenesis observed. In this frame, Biotox® tests were performed to the 250 mg l^{-1} dye solution after anaerobic degradation to assess the acute toxicity of Cibacron Red FN-R intermediates. The EC$_{50}$ parameter was found to be 44 mg l^{-1} C (I control bottle presented no toxicity after a similar treatment), manifesting a significant toxicity increase in relation to the original dye solution (with EC$_{50}$ > 80 mg l^{-1} C). These results would be in agreement with the suggested harmful amines generation hypothesis and, consequently, with the toxic/inhibitory effect to anaerobic biomass. Therefore, since the final aim when treating dye-containing wastewaters is not only decolourisation but also toxicity removal and/or biodegradability improvement, it is clear from the above results that the resulting textile effluents after anaerobic digestion require further post-treatment.

3.3. Aerobic biodegradation post-treatment

250, 1250 and 3135 mg l^{-1} Cibacron Red FN-R anaerobically treated solutions were submitted to Zahn–Wellens assay to investigate the potential of standard aerobic biodegradation as a post-treatment of the anaerobic process. The 1250 mg l^{-1}
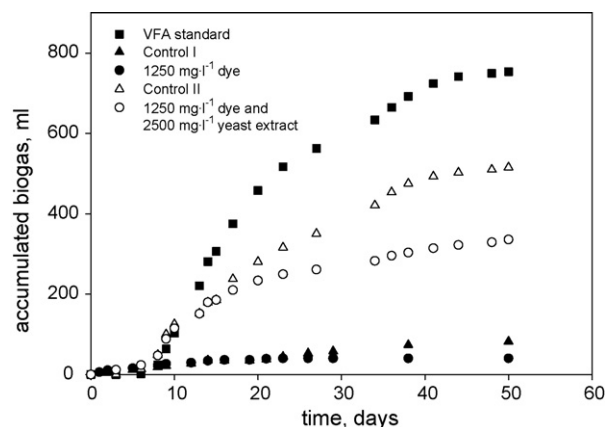

Fig. 1. Accumulated biogas production (1 atm, 37 °C) of the VFA standard and 1250 mg l^{-1} Cibacron Red FN-R solution in presence and absence of 2500 mg l^{-1} yeast extract.
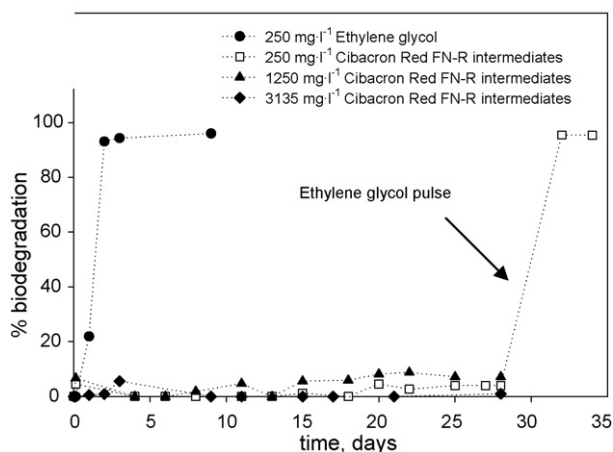
Fig. 2. Zahn–Wellens assay; % biodegradability evolution of 250, 1250 and 3135 mg l^{-1} Cibacron Red FN-R anaerobically treated solutions and the 250 mg l^{-1} standard ethylene glycol.

dye plus 2500 mg l^{-1} yeast extract biodegraded solution (bottle 4) was not considered since the goal was to determine only the azo dye by-products mineralisation. Fig. 2 exhibits that aerobic biomass did not remove substantial DOC within the 28 days of contact (being the biodegradation lower than 9% in all cases), with no sign of cells adaptation. On the contrary, the experiment performed with ethylene glycol, a completely biodegradable standard, achieved 94% biodegradation under the same conditions in just 3 days. The obtained results let us to conclude that Cibacron Red FN-R intermediates after anaerobic digestion (aromatic amines) are of non-biodegradable nature and no further chemical transformation should be expected after aerobic treatment. In agreement with this, Fig. 3 illustrates the similarity between the absorption spectra of the anaerobic and the anaerobic–aerobically degraded 250 mg l^{-1} Cibacron Red FN-R azo dye solution in comparison with the untreated one. Moreover, the EC$_{50}$ parameter was maintained at 42 mg l^{-1} C after the aerobic process providing indirect evidence of no aromatic amines removal. The resistance to aerobic treatment of some aromatic amines generated after anaerobic cleavage of azo dyes has already been reported [16].
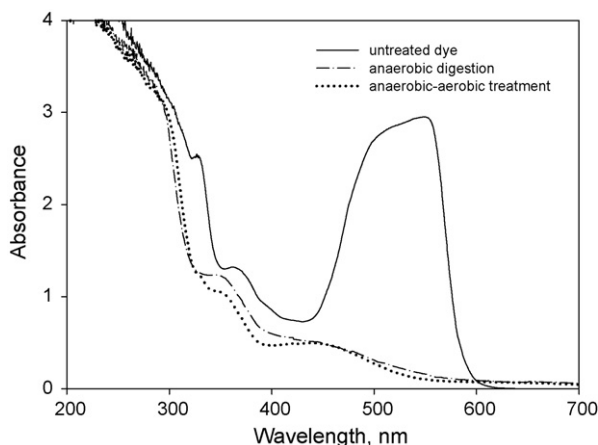
Once the 28 days period was completed, a pulse of ethylene glycol was added to the 250 mg l^{-1} dye intermediates solution in order to detect a possible aerobic biomass inhibition caused by the sample (Fig. 2). In spite of the increased inhibition of luminescence bacteria manifested after the anaerobic process, the standard was consumed in few days demonstrating that the activated sludge was still active or not totally inhibited. These results evidence how Biotox® bacteria are more sensitive to toxicants than activated sludge [27].

3.4. Photo-Fenton and ozonation post-treatments

The solution coming from the anaerobic treatment was submitted to subsequent chemical oxidation by way of photo-Fenton and ozonation processes. The process should eliminate the DOC that could not be removed in the biological unit. The 250 mg l^{-1} Cibacron Red FN-R solution was chosen for these experiments. Anaerobic digestion was repeated four times to obtain the required volume to perform the chemical post-treatment.

For photo-Fenton reaction, the following Fenton reagent combinations were added to aqueous media: 10 mg l^{-1} Fe (II)/250 mg l^{-1} H$_2$O$_2$, 20 mg l^{-1} Fe (II)/500 mg l^{-1} H$_2$O$_2$ and 100 mg l^{-1} Fe (II)/2500 mg l^{-1} H$_2$O$_2$. The evolution of DOC removal percentage versus time is shown in Fig. 4. Results indicate that % DOC removal increased when increasing both Fe (II) and H$_2$O$_2$, although none of the tested dosages were efficient enough to completely remove dye intermediates in a reasonable treatment time. In addition, when varying the Fe (II) dosage from 10 to 100 mg l^{-1}, an increasing DOC removal is first seen (the maximum attained value was 59% with the higher Fenton reagent dosage) followed by a plateau in which no significant DOC evolution was observed. This phenomenon should by attributable to a DOC removal process carried out by iron coagulation since it was accompanied with the appearance of a slight turbidity. The phosphate ion added as a macronutrient during anaerobic stage, which forms insoluble complexes with Fe (III), may be the main cause of iron precipitation. Coagulation was favoured by the high salinity content of the anaerobic effluent. It should be also pointed out that, apart from the photo-Fenton
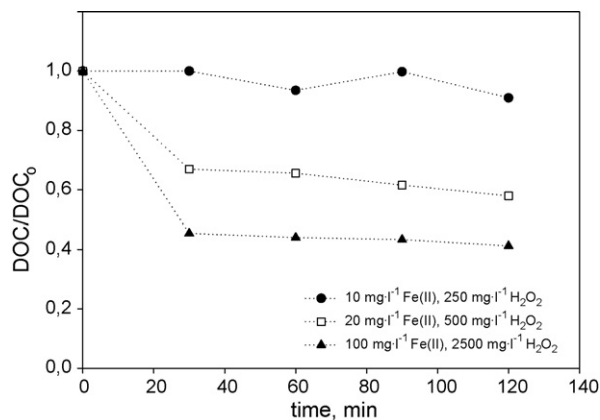

Fig. 3. Absorption spectra of untreated, anaerobic and later aerobically degraded 250 mg l^{-1} Cibacron Red FN-R; pH 7.0.

Fig. 4. DOC/DOC$_o$ evolution versus irradiation time at different Fenton reagent doses for 250 mg l^{-1} anaerobically treated Cibacron Red FN-R; pH 3.0 and $T = 23\,°C$.
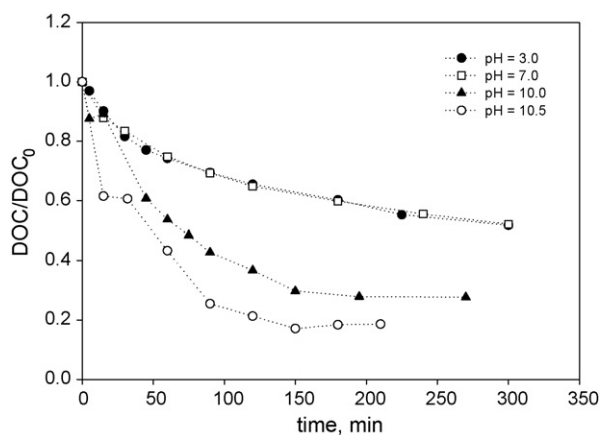
Fig. 5. DOC/DOC$_0$ evolution versus time for 250 mg l^{-1} anaerobically treated Cibacron Red FN-R degradation with ozone at different pH conditions. $T = 23\,^{\circ}$C.

catalyst precipitation, the high salinity content of the anaerobic effluent would also cause a HO$^{\bullet}$ scavenging effect, thus reducing the effectiveness of oxidation. Therefore, to effectively apply the photo-Fenton process, the complex matrix of the resulting anaerobic solution makes necessary a pre-treatment (i.e., phosphate ion removal) or higher Fenton reagent requirements.

Different pH conditions were tested for dye intermediates degradation with ozone: pH 3.0, 7.0, 10.0 and 10.5 (Fig. 5). In each case, the final pH were 2.6, 3.1, 5.1 and 9.8, respectively. The best results were attained at alkaline pH, especially at 10.5, with a substantial 83% mineralisation in 150 min. Both neutral and acid conditions presented the same low degradation (48%). The obtained results are attributable to a different oxidation mechanism depending on pH conditions [28]: for acid or neutral values, the oxidising agent is the molecular ozone in a direct and highly selective attack to aromatic compounds. At alkaline pH, a reaction between ozone and the hydroxide ion give rise to the formation of the HO$^{\bullet}$ radical, which reacts non-selectively with most organic compounds. Accordingly, the free radical mechanism should only be the main one in the pH 10.5 experiments since that is the only case where the pH remains alkaline through the whole reaction. Therefore, not just aromatic but aliphatic compounds generated at the final stage of the process are degraded. Finally, acute toxicity of the resulting solution was determined and EC$_{50}$ was found to be higher than DOC concentration present in solution (EC$_{50}$ > 100%). Therefore, in addition to Cibacron Red FN-R intermediates degradation, ozone AOP has been capable of transforming such compounds into harmless end products.

4. Conclusions

The sequenced anaerobic–aerobic biological treatment was not a plausible alternative for Cibacron Red FN-R reactive azo dye and its intermediates removal. Three different dye concentrations were tested: 250, 1250 and 3135 mg l^{-1}. An almost complete decolourisation took place during the anaerobic stage for all concentrations, with 92–97% effectiveness. However, the resistance of dye intermediates (presumably hazardous aro-

matic amines resulting from reductive cleavage of azo bonds), in addition with the lack of biogas production, evidenced that no methanogenesis occurred under reducing conditions. A possible toxic/inhibitory effect of solutions to anaerobic biomass while increasing dye concentration was observed. Moreover, according to Biotox$^{\circledR}$ and Zahn–Wellens assays, the obtained anaerobic colourless solutions were found to be more toxic than the original dye solution and aerobically non-biodegradable (being the % biodegradation lower than 9 in all cases), impeding the anaerobic–aerobic two-stage treatment for complete degradation.

When applying photo-Fenton and ozonation processes as anaerobic digestion chemical post-treatments, the best results were obtained by means of ozonation at pH 10.5, achieving 83% of global mineralisation and giving place to a final harmless effluent. In contrast, the photo-Fenton catalytic process was not efficient enough to completely oxidise the effluent. Just 59% of DOC removal of the anaerobically treated solution was attained under 100 mg l^{-1} Fe (II)/2500 mg l^{-1} H$_2$O$_2$ photo-Fenton conditions, mostly attributable to a non-oxidative process based on iron coagulation.

In summary, complete Cibacron Red FN-R decolourisation was attained when applying anaerobic digestion though further non-biological processes are required for effective by-products elimination. The % mineralisation achieved by coupling both processes was similar than the obtained in a previously reported photo-Fenton-aerobic biological treatment, which was 80% [15]. These results open the possibility of an anaerobic–chemical sequenced treatment for aerobically non-biodegradable azo dyes removal, being of special interest for real textile wastewater applications.

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