Journal of Hazardous Materials 205–206 (2012) 1–9

Contents lists available at SciVerse ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Assessment of by-products of chlorination and photoelectrocatalytic chlorination of an azo dye

Rafael Leite de Oliveira [a], Marc A. Anderson [c], Gisela de Aragão Umbuzeiro [b], Guilherme Julião Zocolo [a], Maria Valnice Boldrin Zanoni [a,*]

[a] Electroanalytical Chemistry Laboratory, Department of Analytical Chemistry, UNESP, University of São Paulo State, Brazil
[b] Environmental Ecotoxicology and Microbiology Laboratory – LEAL, Faculty of Technology, University of Campinas, Limeira, SP, Brazil
[c] Water Science and Engineering Laboratory 660 North Park Street, Madison, USA

ARTICLE INFO

Article history:
Received 5 August 2011
Received in revised form
23 September 2011
Accepted 21 October 2011
Available online 29 November 2011

Keywords:
CI Disperse Blue 291
Chlorination of dyes
Removal of azo dye
Phoelectrochemical chlorination
Byproducts of chlorination

ABSTRACT

The present work describes a more efficient methodology for the chlorination of water containing disperse dyes, where the chlorinated byproducts identified by mass spectra are compared. For this investigation, we tested the degradation of CI Disperse Blue 291 dye, 2-[(2-Bromo-4,6-dinitrophenyl)azo]-5-(diethylamino)-4-methoxyacetanilide) a commercial azo dye with mutagenic properties. The present work evaluates the photoelectrocatalytic efficiency of removing the CI Disperse Blue 291 dye from a wastewater of the textile industry. We employed NaCl as a supporting electrolyte. It should be noted that photoelectrocatalytic techniques are non-conventional method of generating chlorine radicals. The by-products formed in this process were analyzed using spectrophotometry, liquid chromatography, dissolved organic carbon, mass spectral analysis and mutagenicity assays. The process efficiency was compared with the conventional chlorination process adopted during sewage and effluents treatment processes. This conventional chlorination process is less efficient in removing color, total organic carbon than the photoelectrochemistry technique. Furthermore, we shall demonstrate that the mutagenicity of the generated by-products obtained using photoelectrocatalysis is completely different from that obtained by the conventional oxidation of chloride ions in the drinking water treatment process.

1. Introduction

The occurrence of mutagenic response to contaminants eminating in some textile and wastewater treatment plants processing residuals from textile finishing companies has been reported in the literature [1–5]. The routine backtracking of wastewaters entering treatment plants from the production plants of some textile processing companies has led to the identification of textile dyes as being a cause of the high mutagenic effects impacting the water exiting the treatment plant as well as drinking water quality [6–10].

CI Disperse Blue 291 dye, 2-[(2-Bromo-4,6-dinitrophenyl)azo]-5-(diethylamino)-4-methoxyacetanilide) (Fig. 1) is a commercial azo dye (CAS 51868-46-3) highly used in the textile industry for dyeing polyester. The compound has hydrophobic properties, which can easily adsorbed on aquatic sediments or forms stable suspensions that can be transported by receiving waters and ultimately reach water treatment plants [10]. The presence of CI Disperse Blue 291 and five 2-phenylbenzotriazole (PBTA) derivatives has being detected in extracts obtained from several rivers in Japan that have been receiving effluents from textile dyeing factories. Those dye related compounds have been the main source of the mutagenic activity detected in river waters [11].

CI Disperse Blue 291 was detected as a mutagenic for TA1537, TA1538, TA98, TA100, TA98DNP6, YG1024 and YG1041 strains of Samonella both with and without metabolic activation system (S9) [12,13]. The genotoxicity aspects of aminoazobenzenes dyes have been previously studied [14–17]. The chemical structure analysis involving these dyes showed that the mutagenic responses in Salmonella and mammalian assay systems depend on the nature and position of halogen substituents with respect to both the aromatic rings and the amino nitrogen atom. Because minor changes in the molecule can drastically modify mutagenic activity and carcinogenic potential, it is important that each azo dye released into the market is adequately tested. Among the aminoazobenzene dyes with nitro and halogenated substituents the only dye that has been extensively evaluated for mutagenicity has been the dinitrobromoaminoazobenzene dye CI Disperse Blue 79 [18]. Because of the mutagenic properties of dye compounds, the development of methods to treat and recycle wastewaters containing these compounds is an issue of immediate concerns due to the proven toxicity but yet

* Corresponding author. Tel.: +55 1633019619.
E-mail address: boldrinv@iq.unesp.br (M.V.B. Zanoni).

0304-3894/$ – see front matter © 2012 Published by Elsevier B.V.
doi:10.1016/j.jhazmat.2011.10.060

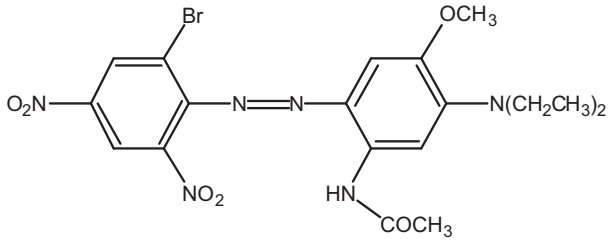


Fig. 1. Chemical structure of the Disperse Blue 291 dye.

the commercial importance of these dyes in the textile industry. The search for new chlorinated disinfection by-products in drinking water has been a challenge. Often, researchers generally have to attempt to identify unknown chlorinated products generated from incompletely defined starting materials, albeit more sophisticated analytical chemical techniques and new assays have helped sort out these chemical species as well as their impact with respect to toxicity [19,20]. Furthermore, we believe that the photoelectrocatalytic process can be used to reduce the presence of undesirable mutagenic compounds.

Photoelectrocatalytic oxidation has being successfully applied for treatment of organic pollutants, to promote oxidation of bromide, chloride and bromate reduction [21–26]. It is accepted that the oxidation process is based mainly on the active oxidants generated at the surface of semiconducting oxides such as the hydroxyl radical ($OH^{\bullet}$), formed due to the oxidation of adsorbed water/OH^- groups, which has powerful oxidation properties ($E^0 = 2.73$ V vs NHE), as shown in Eqs. (1) and (2). Nevertheless, if the photoelectrocatytic oxidation process is conducted in a chloride medium under acidic condition this leads to the formation of active chlorine, generated in situ by direct oxidation of the chloride ion on an electrode based on Ti/TiO_2 under a biasing potential and in the presence of UV irradiation [21–24]. The adsorbed chloride ions on the TiO_2 semiconductor anode surface could be oxidized by holes generated under UV irradiation leading to formation of $Cl^{\bullet}/HOCl^{\bullet}/Cl_2^{\bullet}$ species, as demonstrated in Eqs. (3) and (4). In both cases, whether or not competitive reactions with organic compounds is occurring, there will be consequent formation of active chlorine (Cl_2, HClO, ClO^-) in solution (Eq. (5)).

Photoanode:

$$TiO_2 + h\nu \rightarrow TiO_2\text{–}e^-_{cb} + TiO_2\text{–}h^+_{vb} \quad (1)$$

$$TiO_2\text{–}h^+_{vb} + H_2O_s \rightarrow TiO_2\text{–}OH^{\bullet}_s + H^+ \quad (2)$$

$$TiO_2\text{–}OH^{\bullet}_s + Cl^- \rightarrow HClO^{\bullet} \quad (3)$$

$$TiO_2\text{–}h^+_{vb} + Cl^-_s \rightarrow TiO_2\text{–}Cl^{\bullet}_s \quad (4)$$

$$Cl^{\bullet} + Cl^{\bullet}\,(H^+,\ H_2O)^{\bullet} \rightarrow Cl_2 \quad (5)$$

Cathode (counter electrode):

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (6)$$

Because chorine radicals ($E^0_{Cl^{\bullet}/Cl^-} = 2.410$ V vs NHE and $E^0_{Cl_2/2Cl^-} = 2.090$ V vs NHE) are stronger oxidants than active chlorine ($E^0_{Cl_2/2Cl^-} = 1.395$ V vs NHE), their generation by photoeletrocatalytic condition should be strongly encouraged. We have previous shown that the photoelectrocatalytic process seems to be a good alternative for promoting the formation of active chlorine and thereby accelerating the degradation of dyes due to the formation of $Cl^{\bullet}$ or $Cl_2^{\bullet -}$ radicals as intermediates. These radicals are formed from the direct oxidation of chloride ions on the anode surface irradiated by UV with an applied biasing potential [19]. However, the use of NaCl as supporting electrolyte in the photoelectrocatalytic oxidation of organic compounds has somewhat distrusted as there have been concerns about the formation of possible mutagenic chlorinated by-products due to parallel electrochemical oxidation and/or radical hydroxyl reaction of chloride. Furthermore, chloride ions are naturally present in surface waters at levels of 250 mg L^{-1} and the use of active chlorine is the usual method of disinfection process applied in effluent or in drinking water production. In addition, the use of chloride ions has demonstrated enhancement effect on the photoeletrochemical technology applied to organic pollutants abatement [27–29]. Therefore, it would seem valuable to investigate the effect of chloride ions on both the photoelectrochemical oxidation process as well as the typical chlorination procedure with respect to the generation of mutagenic by-products.

In the present work the photoeletrocatalytic process was applied in the removal of CI Disperse Blue 291 dye from wastewater of textile industry using sodium chloride as supporting electrolyte. The formation of oxidation by-products and the effect on mutagenicity were evaluated. In parallel, conventional chemical chlorination process was investigated in order to comparatively evaluate the formation of chlorination by-products and their mutagenicity.

2. Experimental

2.1. Preparation of Ti/TiO_2 thin-film electrodes

Titanium (IV) isopropoxide (Aldrich) was used as a precursor for preparing TiO_2 colloidal suspensions. Typically, titanium isopropoxide was added to a nitric acid solution keeping the ratio $Ti/H^+/H_2O$ at 1/1.5/200. The resulting precipitate was continuously stirred until completely peptized to a stable colloidal suspension. This suspension was dialyzed against ultrapure water (Milli-Q, Millipore) to a pH of 3.5 by using a Micropore 3500 MW cut off membrane. Thin-film photoelectrodes were dip-coated onto a titanium foil back contact (0.05 or 0.5 mm thick, Goodfellow Cambridge Ltd.), after heating the Ti foils at 350 °C. A sequence of dipping, drying and firing at 450 °C for 3 h was used after each coating (five repetitions).

2.2. Apparatus and procedure

The photoelectrocatalytic oxidation experiments were performed in a 250 mL photoelectrochemical reactor equipped with water refrigeration using an ultra-thermostatic bath (Nova Técnica, Brazil), as shown in Fig. 2. In the cell, was positioned a working electrode, an auxiliary electrode and a Ag/AgCl reference electrode was placed close to the working electrode using a bridge tube containing a Vycor frit tip. The photoactive area of the anode (TiO_2) was 25 cm^2 and was illuminated with a UV light source (254 nm) by using a 125 W Philips medium pressure mercury. This bulb is placed in the center of a quartz sleeve. The distance from the light source to the photoanode is 2.5 cm. The dye and textile wastewater solution was placed in the reactor without any pre-treatment and the photoelectrochemical process was performed under the conditions of bubbling compressed air into the reactor. A Pt gauze electrode was used as counter electrode.

A potentiostat/galvanostat EG & G PARC Model 283 controlled by the electrochemical 270 software was used to bias the photoanode in these photoelectrocatalytic oxidation experiments.

2.3. Dye monitoring

A high performance liquid chromatograph Shimadzu Model 10 AVP coupled with a photodiode array detector was used to separate

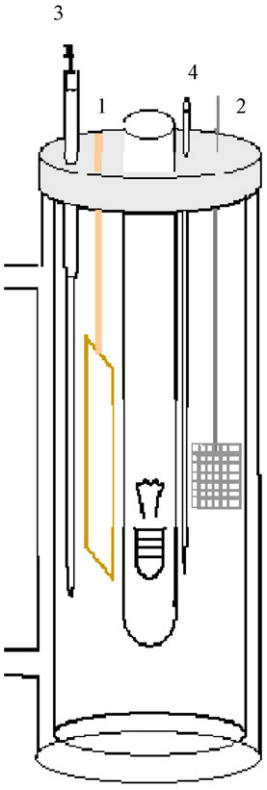


Fig. 2. Photoelectrocatalytic reactor with: (1) photoanode of Ti/TiO_2 illuminated by a commercial lamp (Phillips) of 125 W cm^{-2} operating as a UV light source (315–400 nm) mounted 5 cm in front of the working electrode. A platinum gauze used as the counter-electrode (2) and a saturated calomel electrode (SCE) placed close to the working electrode using a bridge tube containing a Vycor frit tip (3) and (4) air flow.

and identify products and intermediates of these reactions. The separation column was C-18 (4.6 mm × 250 mm, 5 μm) and the mobile phase was acetonitrile:water (85:15) flowing at 1.0 mL min^{-1}. Signals obtained from detector were analyzed by area integration. Standard curves and quantitative analysis of standard Blue 291 dye were carried out by linear regression plotting peak area vs concentration. The procedure was carried out in triplicate for each sample.

Absorption spectra in the ultraviolet and visible range were recorded with a Hewlett-Packard spectrophotometer, model HP 8452A in a 10 mm quartz cell. A total organic carbon analyzer (TOC-VCPN, Shimadzu, Japan) was employed for to study the degree of organic carbon mineralization of dye solution. Prior to injection into the TOC analyzer, the samples were filtered using a 0.45 μm Millipore filter to remove any particles. The measurements are expressed as media of three repetitions.

The original dye and by-products generated by chemical chlorination were analyzed by mass spectrometry using an UltrOTOFQ – ESI-TOF Mass spectrometric Bruker Daltonics, Billerica, MA, USA. This unit was operated under high-resolution internal and external calibration before performing the analysis of samples. The internal calibration solution was Na-TFA 10 mg/mL (TOF). We used an infusion pump with a flow rate of 300 L/h, an end plate set at 4000 V, a 4500 V capillary with the capillary exit at 300 V, 55 V for a Skimmer, a 90 ms transfer, a collision exit gate at 65 ms, dry gas temperature 160 °C, dry gas flow of 4 L min^{-1}, Neb. Gas Pressure was set at 2 bar and the gas used in the measurements was nitrogen. The mobile phase was acetonitrile, and the method of detection was positive for the time of analysis.The products obtained after 120 min of degradation through the photoelectrocatalytic chlorination process were analyzed by mass spectrometry using a model 3200 QTRAP system equipped with a Tubo V Source and Electrospray Ionization – ESI. Samples were infused into the ESI source at flow rates of 10 μL min^{-1} via microsyringe pump. ESI-MS(/MS) experiments were carried out in the Full Scan mode (*m/z* 50–600 Da) and by selection of a specific ion in Q1 and performing its fragmentation using collision-induced dissociation (CID) with nitrogen in the collision chamber.

All the samples were filtered before injection with a syringe in PTFE filters of 0.22 μm (sterile disposable filter unit mark Millipore Millex® model).

The original dye and by-products generated by chemical chlorination were analyzed by mass spectrometry using an UltrOTOFQ – ESI-TOF Mass spectrometric Bruker Daltonics, Billerica, MA, USA. This unit was operated under high-resolution internal and external calibration before performing the analysis of samples. The internal calibration solution was Na-TFA 10 mg/mL (TOF). We used an infusion pump with a flow rate of 300 L/h, an end plate set at 4000 V, a 4500 V capillary with the capillary exit at 300 V, 55 V for a Skimmer, a 90 ms transfer, a collision exit gate at 65 ms, dry gas temperature 160 °C, dry gas flow of 4 L min^{-1}, Neb. Gas Pressure was set at 2 bar and the gas used in the measurements was nitrogen. The mobile phase was acetonitrile, and the method of detection was positive for the time of analysis.The products obtained after 120 min of degradation through the photoelectrocatalytic chlorination process were analyzed by mass spectrometry using a model 3200 QTRAP system equipped with a Tubo V Source and Electrospray Ionization – ESI. Samples were infused into the ESI source at flow rates of 10 μL min^{-1} via microsyringe pump. ESI-MS(/MS) experiments were carried out in the Full Scan mode (*m/z* 50–600 Da) and by selection of a specific ion in Q1 and performing its fragmentation using collision-induced dissociation (CID) with nitrogen in the collision chamber.

All the samples were filtered before injection with a syringe in PTFE filters of 0.22 μm (sterile disposable filter unit mark Millipore Millex® model).

2.4. Chlorination of the CI Disperse Blue 291 dye (CI DB 291)

Solutions of 20 mg L^{-1} of CI Disperse Blue 291 dye were submitted to conventional chemical chlorination with active chlorine gas. The final concentration of the residual free chlorine [18] in the treated water was quantified by colorimetric method based on N,N′-diethyl-p-phenylenediamine (DPD) reaction according to the official method [30].

2.5. Mutagenic activity evaluation

The Salmonella/microsome assay was performed using the treated and non-treated dye solutions. The in situ concentration procedure was applied. Aliquots of a maximum of 2 mL of each sample were diluted in more concentrated top agar [31] with strains of *Salmonella typhimurium* TA98 (*his* D3052, *rfa*, Δ*bio*, Δ*uvr*B, pKM101) and YG1041, a derivative of the TA98, able to produce high levels of nitroreductase and O-acetyltransferase [32]. Exogenous metabolic activation was provided by Araclor 1254 induced Sprague Dawley rat liver S9 mix [33] and necessary cofactors at the concentration of 4% (v/v). Positive controls were 4-nitroquinoline (Acros) at 0.25 μg/plate and 2-aminoanthacene (Sigma/Aldrich) at 2.5 μg/plate for both strains in the absence and presence of metabolic activation S9 mix, respectively. Results were statistically analyzed using the Salanal computer program, with the Bernstein model [34]. Samples were considered positive when significant ANOVA and positive dose response were obtained. $P < 0.05$ was considered statistically significant. The results were expressed as the number of revertants per liter equivalent of each sample

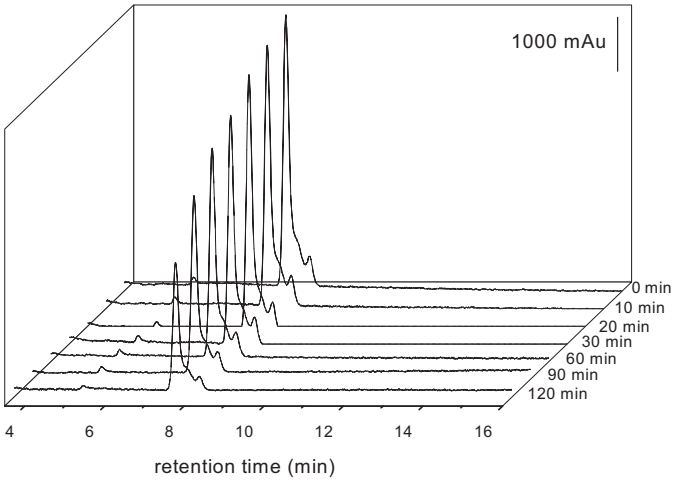


Fig. 3. Diode array chromatograms of a 20 mg L^{-1} solution of Disperse Blue 291 dye before (0 min) and after chemical chlorination (80 mg L^{-1} of active chlorine) during 120 min of reaction. Chromatographic conditions (HPLC-DAD): acetonitrile/water 85:15 as mobile-phase, C_{18} column as stationary-phase, oven temperature 40 °C, flow rate 1.0 mL min^{-1}.

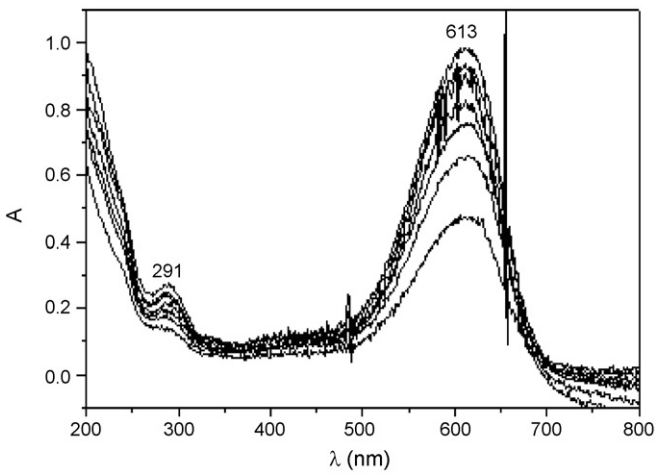


Fig. 4. UV–vis spectra obtained by diode array detection (DAD) under hydrodynamic conditions (HPLC) obtained at 8.1 min of retention time for a solution of 20 mg L^{-1} solution of Disperse Blue 291 dye before (0 min, curve 1) and after 120 min of chemical chlorination (curves 2–7). Chromatographic conditions (HPLC-DAD): acetonitrile/water 85:15 as mobile-phase, C_{18} column as stationary-phase, oven temperature 40 °C, flow rate 1.0 mL min^{-1}.

tested. A blank control solution containing only the electrolytic supporting agent (sodium chloride) was tested. Five doses ranging from 0.05 to 2 mL per plate, which contained different equivalent amounts of the dye or treated dyes, were tested in duplicate plates both in the presence and absence of S9 mix. The doses were expressed in μg equivalent of the dye, except for the blank control. Doses were selected according to sample availability and sensitivity of each strain. The test is based on the detection of histidine-independent revertants in selected Salmonella strains after exposure to mutagens with or without additional activating enzymes. The dose response can be quantified by varying sample concentration and counting revertant colonies per plate at each concentration. Background was carefully evaluated and when there was a reduction of the number of revertants in relation to the negative control or no growth at all, samples were considered as toxic.

3. Results and discussion

3.1. Quantitative analysis of CI Disperse Blue 291 dye

Fig. 3 exhibits the chromatogram obtained for 20 mg L^{-1} disperse Blue 291 dye in Na_2SO_4 0.2 mol L^{-1} at pH 6.0 by HPLC-DAD. This data was obtained by using the best experimental condition of acetonitrile/water 85:15 (v/v), column C-18, T = 40 °C and at a flow rate of 1.0 mL min^{-1}. The chromatograms are characterized by a main peak at a retention time (t_r) of 7.34 min attributed to disperse Blue 291. Characteristic UV–vis spectra obtained by diode array detection under these hydrodynamic conditions was recorded and used as a parameter to identify and confirm the dye. The spectra of the major components presented in the commercial 291 dye are displayed in Fig. 4 (curve 1). The spectra present two bands of absorption at 613, 290 nm, which was attributed to the azo group and other aromatics present in the dye molecule, respectively. The small peak at t_r = 7.95 min is attributed to small quantities of contaminant present in the commercial sample of disperse Blue 291 dye. Taking into account that each substance presents a different absorption pattern, the diode array detection confirmed the presence of these compounds by their UV–vis spectra. After confirmation, we proceeded with the quantitative analysis of the disperse dyes present in the commercial sample.

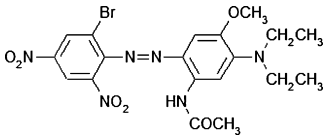
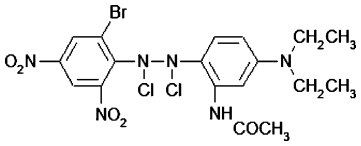
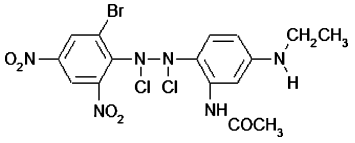
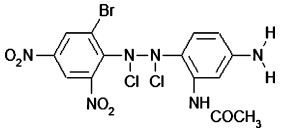
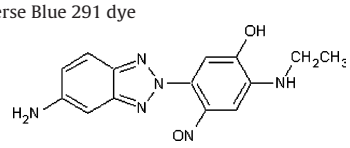
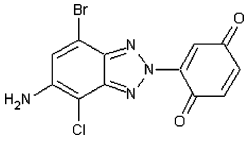
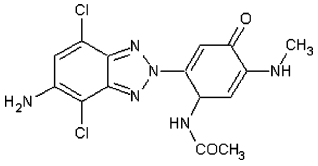
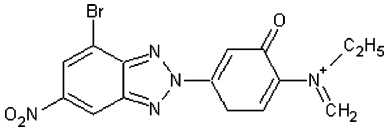
The analytical curves constructed from 1 to 20 mg L^{-1} of disperse Blue 291 dye exhibited an excellent linear relationship from 1 to 11 mg L^{-1}, r = 0.9962, n = 10. The limit of detection (LOD) evaluated as the signal-to-noise ratio equal to 3:1 reaches values of around 0.59 mg L^{-1} and the limit of quantification (LOQ) determined as the signal-to-noise ratio equal to 10:1 was calculated (LOQ = 10 × (SD/B)) and the values are around 1.96 mg L^{-1} were obtained. The repeatability of the proposed method, evaluated in terms of relative standard deviation, was measured as 2.2% over 10 experiments using samples containing 5 mg L^{-1} of the dye.

3.2. Chemical chlorination effects on CI Disperse Blue 291 Dye

To investigate the products generated after treatment by chemical chlorination of this dispersed azo dye, 200 mL of solution containing 20 mg L^{-1} of the commercial blue dye was treated with 80 mg L^{-1} of active chlorine. The curves obtained after 10–120 min of reaction are shown in Fig. 3. The respective UV–vis spectra under hydrodynamic conditions recorded taking the chromatographic peak at t_r = 7.34 min are shown in Fig. 4. Both chromatographic peaks and UV–vis spectra were obtained under chemical chlorination, as described in Section 2. After 2 h of chlorination we observed a 60% decrease of the peak areas attributed to the original dye (t_r = 7.34 min) and contaminant (t_r = 7.95 min). In addition, curves obtained from 10 to 120 min of treatment shown in Fig. 4 indicate that the UV–vis absorbance spectra monitored at the specific retention time of 7.34 min reaches 60% of discoloration after 2 h of the chlorination reaction. The chromatograms also displayed remaining peaks around t_r = 4 min indicating that chemical chlorination is not an effective process for completely removing the dye and also can form by-products not yet identified by the HPLC-DAD conditions employed.

To follow the degradation of Disperse Blue 291 dye by the chlorination process the solution treated during 120 min was analyzed for dissolved total organic carbon (TOC). Only a maximum of 8% of TOC removal was obtained (Fig. 5, curve A). These results suggest that the chlorination process involves only the partial cleavage of the dye molecule. However, there is not a complete mineralization of the product. To obtain more information about the chlorinated by-products, ESI-MS/MS measurements were carried out. Analysis of CI Disperse Blue 291 dye showed

Table 1
MS/MS transition ion scan obtained by LC–MS–MS for CI Disperse Blue 291 dye and its derivatives after chemical chlorination and photoelectrochemical chlorination.

MS/MS transition	(MM)	Chemical structure
Disperse Blue 291 dye		
$[M^+, {}^{79}Br]$ 509	M = 508	
$[M^+, {}^{81}Br]$ 511	M + 2 = 510	
By-products generated after chemical chlorination of CI Disperse Blue 291 dye		
$[M+H]^+$ = 549	M = 548	
$[M+H]^+$ = 520	M = 519	
$[M+H]^+$ = 492	M = 491	
By-product generated after 120 min of photoeletrocatalytic chlorination of CI Disperse Blue 291 dye		
$[M+H]^+$ = 299	M = 298	
$[M+H]^+$ = 353	M = 352	
$[M+H]^+$ = 381	M = 380	
$[M+H]^+$ = 391	M = 390	

two intense peaks with the characteristic m/z ratio of ([M+, ^{79}Br] = 509; [M+2, ^{81}Br] = 511) as shown in Table 1, confirming the pattern described previously for this dye [11]. For the chlorinated by-products, three intense peaks are prominent among several peaks of lower intensity, with the characteristic m/z ratio of ($[M+H]^+$ = 549; $[M+H]^+$ = 520; $[M+H]^+$ = 492), whose chemical structures are shown in Table 1. The peak ($[M+H]^+$ = 549) was attributed to the molecule of CI Disperse Blue 291 dye formed after losing a $-OCH_3$ group, to gain $1H^+$ and two chlorines. The peak $[M+H]^+$ = 520 is formed because of losing one ethyl group ($-CH_2-CH_3$), which becomes the peak $[M+H]^+$ = 492 after losing one more ethyl group ($-CH_2-CH_3$). These results confirm that although the chlorination promotes discoloration, the treatment generated chlorinated by-products, which could be more dangerous than those of the original dye.

3.3. Photoelectrochemical chlorination effect on CI Disperse Blue 291 dye

To verify the efficiency of degradation by using photoelectrocatalytic oxidation of chloride, 200 mL of solution containing 20 mg L^{-1} of CI Disperse Blue 291 dye was treated by the photoelectrocatalytic process in the presence of 0.2 mol L^{-1} NaCl as supporting electrolyte. The pH was adjusted to 4 and the Ti/TiO_2 the anode was biased to +1 V under UV irradiation. This experimental condition leads to formation of around 80 mg L^{-1} of active chlorine, measured by spectrophotometric method, when the dye is not present [19]. The generated products were monitored by HPLC-DAD, UV–vis spectra, TOC removal, mutagenic potency and ESI-MS/MS.

In both Figs. 6 and 7 it is possible to observe the effect of photoelectrocatalytic oxidation on the HPLC-DAD chromatograms

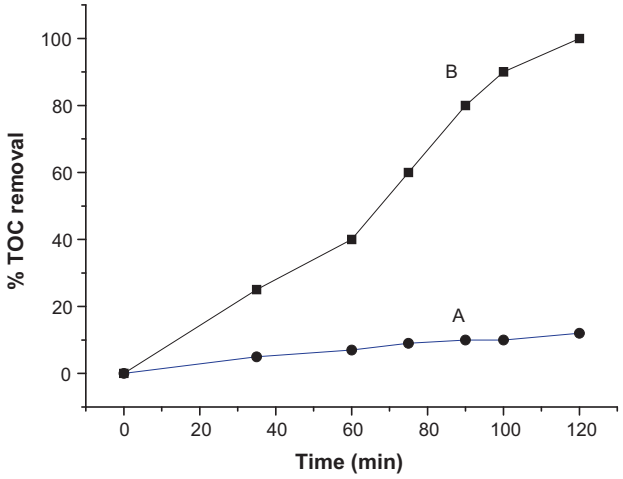


Fig. 5. Total organic carbon (TOC) removal obtained for 20 mg L^{-1} solution of Disperse Blue 291 dye treated with 80 mg L^{-1} of active chlorine (A) and photoelectrocatalytic oxidation on a Ti/TiO_2 anode in NaCl 0.2 mol L^{-1}, pH 6, E = 1 V and UV irradiation (B).

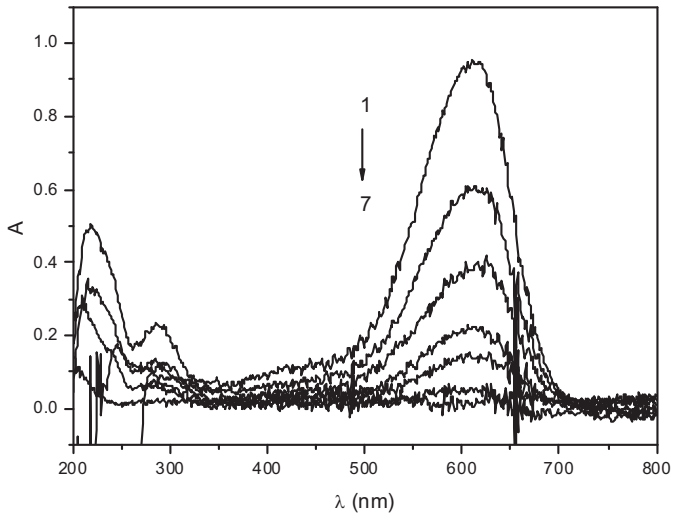


Fig. 7. UV spectra obtained for 20 mg L^{-1} of Dye Blue 291 in solutions of NaCl 0.1 mol L^{-1} on Ti/TiO_2 anodes after photoelectrocatalytic oxidation at +1 V and UV irradiation. Time of photoelectrocatalysis: (1) 0 min; (2) 10 min; (3) 20 min; (4) 30 min; (5) 60 min; (6) 90 min and (7) 120 min. Chromatographic conditions (HPLC-DAD): acetonitrile/water 85:15 as mobile-phase, C_{18} column as stationary-phase, oven temperature 40 °C, flow rate 1.0 mL min^{-1}.

and the UV–vis spectra, respectively obtained during 120 min of treatment of 20 mg L^{-1} of the dye in 0.2 mol L^{-1} NaCl at a pH of 4.0 on these Ti/TiO_2 anodes operating under + 1 V of biasing and subjected to UV irradiation. Fig. 6 indicates that the photoelectrocatalytic oxidation promotes rapid degradation of the dye diagnosed by a suppression of the peak of chromatograms for both dye and contaminant. This degradation process significantly modifies the chemical structure of dye, as shown by the total suppression of the absorbance peaks at both visible and UV region, after 90 min of photoelectrocatalytic process (curve obtained from 10 to 90 min of treatment, Fig. 6). These species completely disappeared after 120 min of photoelectrocatalysis. The degradation follows the equation: $\ln A_0/A = -kt$ (where, A = absorbance of Blue 291 dye at time t, A_0 = absorbance of Blue 291 dye at time = 0 and k = rate constant), which has a rate constant of 0.0691 min^{-1}.

The percentage of dye degradation monitored by dissolved total organic carbon (TOC) removal after photoelectrocatalytic chlorination can be seen in curve B of Fig. 5. For comparison the total organic carbon removal obtained after chemical chlorination is shown in curve A of Fig. 5. The results showed that photoelectrocatalytic oxidation of dye in chloride medium promoted around 100% of TOC removal.

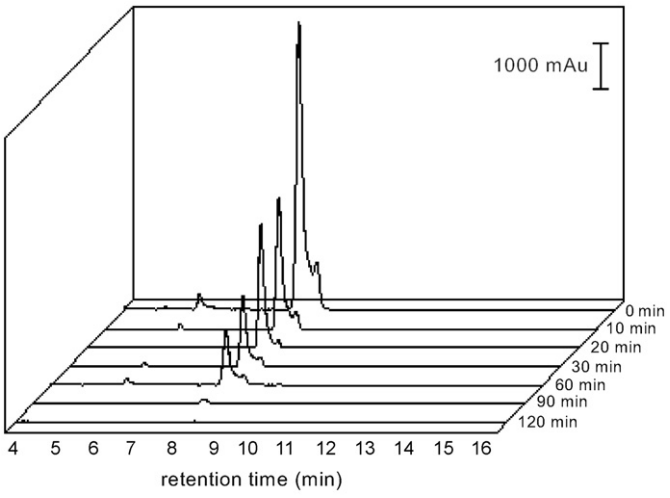


Fig. 6. Chromatograhic profile of 20 a mg L^{-1} of Disperse Blue 291 dye in NaCl 0.2 mol L^{-1} pH 4 before (0 min) and after 120 min of photoelectrocatalytic oxidation on Ti/TiO_2, E = 1 V and UV irradiation. Chromatographic conditions (HPLC-DAD): acetonitrile/water 85:15 as mobile-phase, C_{18} column as stationary-phase, oven temperature 40 °C, flow rate 1.0 mL min^{-1}.

To investigate the products generated after photoelectrocatalytic chlorination of CI Disperse Blue 291 dye, a solution of 20 mg L^{-1} dye was submitted to treatment in 0.2 mol L^{-1} NaCl pH 4.0 on Ti/TiO_2 anodes operating under +1 V and UV irradiation. Aliquots of the final solution were collected after 120 min of treatment. The results analyzed by mass spectrometry using an UltrOTOFQ – ESI-TOF (a technique employed to analyze the results of conventional chlorination) does not shown any significant signal, confirming the TOC results showing 100% removal by photoelectrocatalytic chlorination. Nevertheless, the same sample after extraction (Section 2) was analyzed by ESI-MS/MS equipped with a Tubo V Source and Electrospray Ionization – ESI as described in Section 2. Although the TOC measurement indicated that the dye was totally mineralized after 120 min the mass spectra showed main peaks with an m/z ratio of $[M+H]^+$ = 299 $[M+H]^+$ = 353, $[M+H]^+$ = 381, $[M+H]^+$ = 391. This result is due to the high sensitivity of the applied analytical technique. The chemical structures of the generated products are shown in Table 1. All proposed structures were confirmed by MS^2 spectra. The products seem to be a benzotriazole derivative as shown in Table 1, similar to the one already described by Watanabe et al. [11]. Interestingly, the structures (Table 1) of the ions mentioned above suffered loss of resonance characteristics due to photoelectrocatalyic chlorination after 120 min. The aromatic ring conjugated to the amine and amide groups of the original molecule could be a strong indication that these amide groups and aromatic amine may be likely responsible for the mutagenic character of the molecule. Nevertheless, this behavior could be attributed to the low concentration of these generated by-products upon photoelectrocatylic oxidation. These results strongly support that the mechanism of azo dye degradation is different when photoelectrocatalytic chlorination and chemical chlorination are applied.

3.4. Mutagenicity of the treated CI Disperse Blue 291 solutions

The supporting electrolytic solution containing 0.2 mol L^{-1} sodium chloride (blank solution) was tested in the Salmonella/microsome assay with TA98 and YG1041 in the

Table 2
Mutagenicity data for the supporting electrolytic solution containing 0.2 mol L^{-1} NaCl (blank control) tested with the TA98 and YG1041 strains of Salmonella in the presence and absence of exogenous metabolic activation (S9).

Doses	Mean of number of revertants/plate and standard deviation (SD)							
mL solution/plate	TA98				YG1041			
	−S9		+S9		−S9		+S9	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Negative control	21.7	1.5	23.3	1.7	85.0	3.7	81.6	8.3
0.01					78.5	7.8	80.5	0.7
0.05	22.0	1.4	29.5	3.5	89.0	12.7	73.5	6.4
0.10	21.0	1.4	26.5	4.9	97.0	1.4	86.5	35
0.25	22.0	2.8	27.5	0.7	87.5	10.6	84.0	9.9
0.50	21.5	2.1	26.0	0.00	83.0	5.7	88.0	14.1
1.00	25.5	4.9	23.0	2.8	88.0	11.3	86.5	4.9
2.00	23.5	0.7	24.5	4.9				
Revertants per μg	ND[a]		ND		ND		ND	

[a] Mutagenic activity not detected under the tested conditions.

Table 3
Mutagenicity data for the CI Disperse Blue 291 solution containing in 0.2 mol L^{-1} NaCl tested with the TA98 and YG1041 strains of Salmonella in the presence and absence of exogenous metabolic activation (S9).

Doses	Mean of number of revertants/plate and standard deviation (SD)							
μg equivalent of dye/plate	TA98				YG1041			
	−S9		+S9		−S9		+S9	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Negative control	21.7	1.5.	23.3	1.7	85.0	3.7	81.6	8.3
0.08					83.50	13.4	68.5	4.9
0.42	17.0	1.4	22.0	2.8	104.5	14.9	69.0	12.7
0.83	21.0	0.0	22.5	0.7	93.5	12.0	84.0	0.0
2.10	14.0	0.0	26.5	2.1	103.0	8.5	110.0	5.66
4.20	19.5	2.1	18.5	0.7	94.5	7.8	171.0	12.7**
8.30	23.0	4.2	28.0	1.4*	135.5	3.54**	300.0	8.5**
16.6	26.5	2.1	33.0	1.4**				
Potency revertants per μg	ND[a]		0.6		4.9		21	

* Significant at 5%.
** Significant at 1%.
[a] Mutagenic activity not detected under the tested conditions.

presence and absence of S9 and provided negative as expected (Table 2). Mutagenicity of the solution containing the CI Disperse Blue 291 provided positive results for both strains as expected (Table 3). YG1041 was more sensitive because it presents high levels of nitroreductase and acetyltranferase that biotransforms the original dye in high mutagenic species. The tests performed for the traditional strains TA98 with and without metabolic activation (S9) indicated that the dye Disperse Blue 291 is mutagenic only when undergoing metabolic activation through S9. In addition, there are considerably increased of mutagenic potency with strain YG1041 with S9. These results illustrate the importance of acetylation and nitroreduction in the mutagenicity of the DB291 dye, since this strain produces large amounts of nitroredutase and acetyltransferase. The presence of nitroredutase in the YG1041 strain could be promoting the reduction of the nitro group of the dye giving rise to the hydroxylamines. On the other hand

Table 4
Mutagenicity data for the CI Disperse Blue 291 solution after chlorination in 0.2 mol L^{-1} NaCl tested with the TA98 and YG1041 strains of Salmonella in the presence and absence of exogenous metabolic activation (S9).

Doses	Mean of number of revertants/plate and standard deviation (SD)							
μg equivalent of dye/plate	TA98				YG1041			
	−S9		+S9		−S9		+S9	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Negative control	21.67	1.5	23.3	1.7	85.0	3.7	81.6	8.3
0.08					87.5	6.4	81.5	4.9
0.42	23.00	4.2	23.0	4.2	80.5	0.7	78.0	0.0
0.83	22.50	3.5	24.0	1.4	83.5	3.5	78.0	2.8
2.10	36.50	2.1**	23.5	2.1	82.5	0.7	104.5	6.4*
4.20	TOX[a]		TOX		TOX		TOX	
8.30	TOX		TOX		TOX		TOX	
16.6	TOX		TOX					
Potency revertants per μg	TOX		TOX		TOX		TOX	

* Significant at 5%.
** Significant at 1%.
[a] Presence of toxicity.

Table 5
Mutagenicity data for CI Disperse Blue 291 solution after 120 min-treatment with photoelectrocatalytic oxidation in 0.2 mol L^{-1} NaCl tested with the TA98 and YG1041 strains of Salmonella in the presence and absence of exogenous metabolic activation (S9).

Doses	Mean of number of revertants/plate and standard deviation (SD)							
μg equivalent of dye/plate	TA98				YG1041			
	−S9		+S9		−S9		+S9	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Negative control	21.7	1.5	23.3	1.7	85.0	3.7	81.6	8.3
0.08					83.5	10.6	66.5	2.1
0.42	20.0	0.0	22.0	2.8	78.0	11.3	83.5	2.1
0.83	18.0	1.4	20.0	0.7	78.5	9.2	86.0	4.2
2.10	25.0	4.2	18.5	7.8	91.5	0.7	82.5	9.2
4.20	17.5	2.1	23.0	1.4	98.0	21.2	76.5	16.3
8.30	21.5	2.1	19.0	7.1	93.0	0.0	64.0	11.3
16.6	22.0	0.0	20.5	0.7				
Potency revertants per μg	ND[a]		ND		ND		ND	

[a] Mutagenic activity not detected under the tested conditions.

O-acetylated products generated by acetyltransferase, generate highly reactive species, hence the increase in mutagenic potency in strain YG1041 detected for DB291 dye.

Mutagenicity data for the chlorination of the dye solution are shown in Table 4. The results indicated that chlorination produced several by-products that could be also mutagenic (Table 1). However, because toxic by-products were also formed, it was not possible to analyze the effect of the treatment with respect to mutagenicity. This behavior is expected since the compounds formed after conventional chemical chlorination is identified as organochlorinated compounds, as shown in Table 1. Mutagenic/toxic by-products were already observed in other studies [8,35]. The dye solution treated with photoelectrocatalysis chlorination process in 0.2 mol L^{-1} NaCl for 120 min completely removed mutagenicity (Table 5) corroborating the TOC data. This could be attributed to the absence of dinitro groups and azo group in the dye submitted to phoelectrocatalytic treatment, as shown the chemical structure presented in Table 1 identified by mass spectra. The same photoelectrocalytic treatment process was also performed using 0.2 mol L^{-1} sodium sulfate instead of sodium chloride and results for mutagenicity were also negative (data not shown). It is clear that photoelectrocatalytic process was more efficient in the removal of the mutagenicity of the CI Disperse Blue 291 and this process does not generate toxic by-products as in typical chlorination.

4. Conclusions

CI Disperse Blue 291 dye was almost completely mineralized and its mutagenicity was removed under optimized conditions of photoelectrocatalysis using an irradiated and biased Ti/TiO_2 photoanode, using both NaCl as sodium sulfate as supporting electrolytic solution in a short time scale treatment (120 min). Although intermediate compounds can be formed during photoelectrocatalytic chlorination by using the NaCl as supporting electrolytic solution, the occurrence of radical species generated during the photoelectrocatalytic process is a source of powerful radical species that increase oxidation efficiency.

References

[1] A. Oguri, T. Shiozawa, Y. Terao, H. Nukaya, J. Yamashita, T. Ohe, T. Sawanishi, T. Katsuhara, K. Sugimura, K. Wakabayashi, Identification of a 2-phenylbenzotriazole (PBTA)-typemutagem, PBTA-2, in water from the Nishitakase River in Kyoto, Chem. Res. Toxicol. 11 (1998) 1195–1200.
[2] S. Knasmüller, E. Zöhrer, E. Kainzbauer, H. Kienzl, B. Colbert, G. Lamprecht, R. Schulte-Hermann, Detection of mutagenic activity in textiles with *Salmonella typhimurium*, Mutat. Res. 299 (1993) 45–53.
[3] I. Jäger, Research Feasibility Study – Report to the European Commission, March 1998.
[4] OSPAR Commission, Survey of Genotoxicity Test Methods for the Evaluation of Waste Water within Whole Effluent Assessment, 2002, ISBN 1-904426-02-6, www.ospar.org.
[5] K. Schneider, C. Hafner, I. Jäger, Mutagenicity of textile dye products, J. Appl. Toxicol. 24 (2004) 83–91.
[6] G.A. Umbuzeiro, H. Freeman, S. Warren, D.P. Oliveira, M. Sakagami, T. Watanabe, L.D. Claxton, Contribution of azo dyes to the mutagenic activity detected in the Cristais rivers waters, Chemosphere 60 (2005) 55–64.
[7] G.A. Umbuzeiro, D. Roubicek, C.M. Rech, M.I. Sato, L.D. Claxton, Investigating the sources of the mutagenic activity found in a river using the Salmonella assay and different water extraction procedure, Chemosphere 54 (2003) 1589–1597.
[8] D.P. Oliveira, P.A. Carneiro, C.M. Rech, M.V.B. Zanoni, L.D. Claxton, G.A. Umbuzeiro, Mutagenic compounds generated from the chlorination of disperse azo-dyes and their presence in drinking water, Environ. Sci. Technol. 40 (2006) 6682–6689.
[9] D.P. Oliveira, P.A. Carneiro, M.M. Sakagami, M.V.B. Zanoni, G.A. Umbuzeiro, Chemical characterization of a dye processing plant effluent – identification of the mutagenic components, Mutat. Res. 626 (2007) 135–142.
[10] T. Ohe, T. Watanabe, K. Wakakayashi, Mutagens in surface water, Mutat. Res. 567 (2004) 109–149.
[11] T. Watanabe, T. Shiozawa, Y. Takahashi, T. Takahashi, Y. Terao, H. Nukaya, T. Takamura, H. Sawanishi, T. Ohe, T. Hirayama, K. Wakabayashi, Mutagenicity of two 2-phenylbenzotriazole derivatives, 2-[2-(acetylamino)-4-(diethylamino)-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2H-benzotriazole and 2-[2-(acetylamino)-4-(diallylamino)-5-methoxyphenyl]-5-amino-7-bromo-4-chloro-2H-benzotriazole and their detection in river water in Japan, Mutagenesis 17 (2002) 293–299.
[12] G.A. Umbuzeiro, H. Freeman, S.H. Warren, F. Kummrow, L.D. Claxton, Mutagenicity evaluation of the commercial product CI Disperse Blue 291 using different protocols of the Salmonella assay, Food Chem. Toxicol. 43 (2005) 49–56.
[13] J.A. Miller, E.C. Miller, The carcinogenity of 3-methoxi-4-aminoazo-benzene and its N-methyl derivatives for extrahepatic tissues of the rat, Cancer Res. 21 (1961) 1068–1074.
[14] T. Yahagi, M. Degawa, Y. Seino, T. Matsushima, M. Nagao, T. Sugimura, Y. Hashimoto, Mutagenicity of carcinogenic azo dyes and their derivatives, Cancer Lett. 1 (1975) 91–96.
[15] M. Degawa, S. Miyairi, Y. Hashimoto, Eletrophilic reactivity and mutagenicity of ring-methyl derivatives of N-acyloxy-Naminoazobenzene and related azo dyes, Gann 69 (1978) 367–374.
[16] J. Ashby, P.A. Lefreve, R.D. Callander, The possible role of azoreduction in the bacterial mutagenicity of 4-dimethylaminoazobenzene (DAB) and 2 of its analogues (6BT and 5I), Mutat. Res. 116 (1983) 271–279.
[17] J.F. Esancy, H.S. Freeman, L.D. Claxton, The effect of alkoxy substituents on the mutagenicity of some aminoazobenzene dyes and their reductive-cleavage products, Mutat. Res. 238 (1990) 1–22.
[18] Federal Register, C.J. Disperse Blue 79:1; Testing Consent Order. Rules and Regulations, 1989.
[19] M.E. Osugi, G.A. Umbuzeiro, F.J. De Castro, M.V.B. Zanoni, Photoelectrocatalytic oxidation of remazol turquoise blue and toxicological assessment of its oxidation products, J. Hazard. Mater. B 137 (2006) 871–877.
[20] P.A. Carneiro, G.A. Umbuzeiro, D.P. Oliveira, M.V.B. Zanoni, Mutagenic activity removal of selected disperse dye by photoelectrocatalytic treatment, J. Appl. Electrochem. 40 (2010) 485–492.
[21] M.V.B. Zanoni, J.J. Sene, M.A. Anderson, Photoelectrocatalytic degradation of Remazol Brilliant Orange 3R on titanium dioxide thin-film electrodes, J. Photochem. Photobiol. A: Chem. 157 (2003) 55–63.
[22] P.A. Carneiro, M.E. Osugi, J.J. Sene, M.A. Anderson, M.V.B. Zanoni, Evaluation of color removal and degradation of a reactive textile azo dye on nanoporous TiO_2 thin-film electrodes, Electrochim. Acta 49 (2004) 3807–3820.

[23] M.E. Osugi, G.A. Umbuzeiro, M.A. Anderson, M.V.B. Zanoni, Degradation of metallophtalocyanine dye by combined processes of electrochemistry and photoelectrochemistry, Electrochim. Acta 50 (2005) 5261–5269.
[24] L.E. Fraga, M.L.P. Beatriz, M.A. Anderson, F.M.M. Paschoal, M.V.B. Zanoni, Evaluation of the photoelectrocatalytic method for oxidizing chloride and simultaneous removal of microcystin toxins in surface waters, Electrochim. Acta 54 (2009) 2069–2076.
[25] H. Selcuk, J.J. Sene, M.V.B. Zanoni, H.Z. Sarikaya, M.A. Anderson, Behavior of bromide in the photoelectrocatalytic process and bromine generation using nanoporous titanium dioxide thin-film electrodes, Chemosphere 54 (2004) 969–974.
[26] F.M.M. Paschoal, G. Pepping, Zanoni Greg, M.A. Anderson, M.V.B. Zanoni, Photoelectrocatalytic removal of bromate using Ti/TiO coated as a photocathode, Environ. Sci. Technol. 43 (2009) 7496–7502.
[27] G. Li, T. Ana, C.J. Chena, G. Sheng, J. Fua, F. Chena, S. Zhang, H. Zhao, Photoelectrocatalytic decontamination of oilfield produced wastewater containing refractory organic pollutants in the presence of high concentration of chloride ions, J. Hazard. Mater. B 138 (2006) 392–400.
[28] W. Zhang, T. An, X. Xiao, J. Fu, G. Sheng, M. Cui, G. Li, Photoelectrocatalytic degradation of reactive brilliant orange K-R in a new continuous flow photoelectrocatalytic reactor, Appl. Catal., A 255 (2003) 221–229.
[29] G. Li, T. An, X. Nie, G. Sheng, X. Zeng, J. Fu, Z. Lin, E.Y. Zeng, Mutagenicity assessment of produced water during photoelectrocatalytic degradation, Environ. Toxicol. Chem. 26 (2007) 416–423.
[30] J.O. Callaway, in: L.S. Clesceri, A.E. Greenberg, R.R. Trussel (Eds.), Standard Methods for the Examination of Water and Wastewater, 17th ed., Washington, DC, 1989, Part 4000, 62.
[31] Coriel Institute for Medical Research, Ames Salmonella Mutagenicity Assay Protocol, Department of Microbiology, CIMR, Camden, NJ, 1986.
[32] Y. Hagiwara, M. Watanabe, Y. Oda, T. Sofuni, T. Nohmi, Specificity and sensitivity of *Salmonella typhimurium* YG1041 and YG1042 strains possessing elevated levels of both nitroreductase and acetyltransferase activity, Mutat. Res. 291 (1983) 171–180.
[33] D.M. Maron, B.N. Ames, Revised methods for the Salmonella mutagenicity test, Mutat. Res. 113 (1983) 173–215.
[34] L. Bernstein, J. Kaldor, J. McCann, M.C. Pike, An empirical approach to the statistical analysis of mutagenesis data from Salmonella test, Mutat. Res. 97 (1982) 267–281.
[35] T. Robinson, G. McMullan, R. Marchant, P. Nigam, Remediation of dyes in textile effluent: a critical review on current treatment technologies with a proposed alternative, Bioresour. Technol. 77 (2001) 247–255.