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ORIGINAL PAPER

Gliding arc discharge-assisted biodegradation of crystal violet in solution with *Aeromonas hydrophila* strain

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Abstract The gliding arc discharge, which is a source of nonthermal plasma, was used to enhance the biodegradation of crystal violet (CV), a triphenylmethane non-biodegradable organic dye. The determination of the biodegradability index, i.e., biochemical oxygen demand (BOD_5)/chemical oxygen demand (COD) ratio, and the total organic carbon measurement were used to assess the biodegradability. For the biological treatment alone, a bacterial strain of *Aeromonas hydrophila* (8×10^8 - CFU mL^{-1}) bleached 42 % of CV solution (50 mg L^{-1}) after 12-h incubation. The bleaching rate was enhanced by increasing the initial bacterial concentration; however, a drop in the bleaching rate was noted when CV concentration was increased. For the plasma process alone, a 15-min treatment resulted in a color removal of 49.7 %, at a mineralization rate of 12.2 %, thereby increasing the BOD_5/COD ratio from 0.11 to 0.23. There was an increase in the bleaching rate in temporal post-discharge conditions (i.e., self-continuity of reaction after the discharge was switched off): For 2 h of temporal post-discharge reaction, the color removal of the 15-min plasma-pre-treated CV increased to 55 %. The disappearance of color during each treatment method followed the first-order kinetics. With regard to the combined plasma/biological treatment process, the 15-min plasma-pre-treated sample was bleached at 92 % by *A. hydrophila* after 2-h incubation and completely bleached for 6 h. Therefore, there is a positive synergism of bacterial and plasma treatments. This combined treatment is useful in reducing the energy involved in complete mineralization of wastewater containing non-biodegradable dyes.

Keywords *Aeromonas hydrophila* · Biodegradability enhancement · Bleaching · Coupling treatment system · Crystal violet · Glidarc plasma

Introduction

It is well known that organic dyes are a source of serious environmental contamination; approximately 10 % of dyes used in industries are found in effluents (Young and Yu 1997). The removal of dyes from wastewaters is currently the subject of considerable investigation. Numerous techniques (physical, chemical, and biological/biochemical, or a combination of two or more techniques) have been reported for the treatment of wastewater contaminated by organic dyes. The purpose of this paper was the application of a new coupling method to the degradation of crystal violet (CV) molecule, a triphenylmethane (TPM) dye, selected as recalcitrant non-biodegradable organic model. CV is used in human and veterinary medicine as biological stains. As other TPM dyes, CV is also involved in paper, food, cosmetic, and

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rubber industries, and for coloring of oils, lipids, varnished plastics (Chiing-Chang et al. 2007; Mittal et al. 2010; Parshetti et al. 2011). Due to its high aromatic content, which stabilizes the molecule, CV is poorly metabolized by microorganisms and has a long life in the environment (Moturi and Singaracharya 2009).

Effluents which have been polluted by CV could be treated efficiently by the use of classical chemical and physiochemical methods (precipitation, coagulation/flocculation, and adsorption). Nevertheless, they are not destructive, given that they transfer organic dyes from water to another phase, thus causing sometimes secondary pollution. Recently, an excellent overview on chemical treatment technologies for wastewater recycling was brought forth, and many methods were critically examined (Gupta et al. 2012a). In fact, biological/biochemical processes (biodegradation) can be used: they are less expensive, reliable, and produce less sludge. For example, microorganisms such as *Agrobacterium radiobacter*, *Pseudomonas putida*, *Bacillus cereus*, and *Aeromonas hydrophila* could degrade CV (Chiing-Chang et al. 2007; Deng et al. 2008; Ren et al. 2006a; Parshetti et al. 2011). Nevertheless, due to the poor biodegradability of TPM dyes, the biological process requires a relatively long incubation time (up to 86 h). Mineralization remains incomplete after the process, and organic products are still obtainable at the end of the treatment (Parshetti et al. 2011). Hence, biological treatment processes used alone against textile effluents do not always yield satisfactory results.

The advanced oxidation processes (AOPs), which are based on the generation of active hydroxyl radicals $^{\cdot}OH$, are claimed to degrade numerous organic compounds (Mantzavinos and Psillakis 2004; Gupta et al. 2012b; Saleh and Gupta 2012; Arab et al. 2015). The gliding arc discharge (glidarc), a convenient source of nonthermal plasma, belongs to these AOPs: It operates close to atmospheric pressure and at room temperature. This plasma exhibits characteristics of both thermal and nonthermal plasma, thereby making interaction with matter similar to that of "quenched" plasma (Lesueur et al. 1988). With humid air as the working gas, $^{\cdot}OH$ and $NO^{\cdot}$ were identified and quantified by spectroscopy measurements as the principal radical species present in the glidarc plasma plume (Benstaali et al. 2002). Moreover, they are precursors of other active species (Brisset et al. 2008). The glidarc technique was successfully tested for the pollutant abatement of aqueous effluents, and several examples relevant to the elimination of dyes found in domestic and industrial wastewaters are reported in the literature: These include TPM dyes such as malachite green, CV, bromothymol blue, and bromophenol blue (Abdelmalek et al. 2006; Doubla et al. 2008; Djepang et al. 2014).

The aim of this study was to test a promising alternative for wastewater treatment: a combination of the biological/biochemical process (an inexpensive method, which is not efficient enough when solutes are non-biodegradable) and glidarc (source of oxidizing species, which can be expensive if the treatment times become long). Indeed, a chemical pre-treatment of an AOP [mainly due to the production of powerful oxidizing agents such as $^{\cdot}OH$ $(E^{\cdot}_{(^{\cdot}OH/H_2O)} = 2.8\,V/SHE)$] may convert recalcitrant organic compounds to biodegradable intermediates, which are subsequently treated biologically; thus, the associated costs are reduced. For example, the photo-Fenton process has been combined with biological treatment to treat water polluted by pesticides (Vilar et al. 2012), and to improve the biodegradability index of organic effluents in order to enhance the efficiency of biochemical treatment (Asha et al. 2014). Lotio et al. (2014) were concerned about the ozonation process coupled with biological process to remove color, COD, and total nitrogen of textile yarn dyeing effluents. To the best of our knowledge, there is no report of attempts to use glidarc plasma/biodegradation coupling for the bleaching and degradation of organic pollutants.

As literature reports that CV solutions could be bleached in the presence of bacteria and independently by exposing the dye solutions to glidarc plasma, the purpose of this paper was to verify the possibility of enhancing the biodegradability of CV by glidarc plasma. Subsequently, plasma-pre-treated wastewater was treated biologically by the *A. hydrophila* strain, which was selected as biological agent.

The glidarc treatment of solutions also favors the occurrence of post-discharge phenomena (i.e., a self-development of the chemical reactions in the solution after the discharge is switched off). Indeed, some of the active oxidizing species are long life water-soluble moieties and are responsible for temporal post-discharge reactions (TPDR) which develop in the target liquid isolated from the plasma reactor (Brisset et al. 2008). A typical example of TPDR is reported during the plasma-chemical treatment of methyl orange dye (Moussa et al. 2007). To avoid any confusion, the degradation of CV in post-discharge conditions was also investigated and compared to the coupling plasma/biological treatment.

This research was carried out between June 2013 and December 2014 at the Laboratory of Mineral Chemistry and the Hydrobiology and Environment Research Unit of University of Yaoundé I, Cameroon. Some analyses were

carried out at the Laboratory of Experimental Bacteriology of Centre Pasteur du Cameroun.

Materials and methods

Reagents

Crystal violet solution

The selected dye, i.e., CV, is a classical TPM dye, i.e., tris(4-(dimethylamino)phenyl)methylium chloride ($[(H_3C)_2N–(C_6H_4)]_2C=(C_6H_4)=N^+(CH_3)_2, Cl^-$), also referred as CV or methyl violet 10 B. The reagent was obtained from Sigma-Aldrich (France) and used without further purification. In aqueous solution, the CV (pH 5.4) has a blue–violet color with a maximum absorption band at 585 nm. At pH 1, CV solution is green with maximum absorption bands at 420 and 620 nm. The pH of the samples was adjusted with H_2SO_4 and NaOH solutions 1 M. The chemical oxygen demand (COD), the biological oxygen demand (BOD_5), and the total organic carbon (TOC) values of the initial solution (50 mg L^{-1}) were 170, 20, and 41 mg L^{-1}, respectively.

Microorganism strain, culture condition, and preparation of suspensions

The bacterial strain, *A. hydrophila*, is an anaerobic facultative and gram-negative bacterium. It was isolated from well water using the membrane filtration technique on ampicillin dextrin agar medium (Marchal et al. 1991; Clesceri et al. 2005). *A. hydrophila* was used in the stationary growth phase; it was cultured using the laboratory method (Lontsi et al. 2013). Briefly, using a platinum loop, bacterial colonies were removed from the test tube and introduced into 10 mL of sterilized NaCl 0.15 M solution, followed by homogenization with a vortex. This suspension was centrifuged twice at 8000 r/min for 10 min at 10 °C. The pellet was re-suspended in NaCl solution 0.15 M. A volume of 1 mL of this bacterial suspension was introduced into 10 mL of sterilized peptone solution and incubated for 17 h at 37 °C in static conditions; at this stage, *A. hydrophila* was in the stationary growth phase. Plate count agar (PCA) was used for the pre-culture; the nutrient broth was tryptic soy broth (TSB); and ampicillin dextrin agar (ADA) medium was used for the cell culture and enumeration.

After the culture, the bacterial cells were harvested by centrifugation at 8000 r/min for 10 min at 10 °C and re-suspended in NaCl solution 0.15 M. Suspensions for the biodegradation experiments were obtained by adjusting the absorbance (or former optical density (OD)) at 600 nm to the desired values. The concentration of each bacterial suspension was determined by the counting method after culture on ADA medium (Rodier et al. 1996). Thus, each OD corresponds to a bacterial concentration in CFU mL^{-1}.

Biological treatment

A volume of 1 mL of the bacterial suspension 0.8 × 10^9 - CFU mL^{-1} was inoculated into sterile 25-mL test tubes containing 10 mL of nutrient broth and 1 mL of CV (50 mg L^{-1}), and incubated at 37 °C in static conditions. For various incubation times (0, 3, 5, 6, 9, 12 h), the tubes were removed from the incubator and centrifuged at 8000 r/min for 10 min at 10 °C to separate the bacterial biomass. The supernatant was used to study the bleaching of CV by measuring its absorbance at the maximum absorption wavelength (λ_{max} = 585 nm) with a spectrophotometer of mark Spectro PC SN 400216. The concentration of CV (mg L^{-1}) in the supernatant was determined from the calibration curve of the CV. Two types of controls were used: uninoculated sterile control and heat-killed control. The former, containing only nutrient broth, indicated the effect of medium components in decolorization, and the latter showed adsorption to cells. Heat-killed bacteria were obtained by autoclaving the bacterial suspensions at 120 °C for 15 min. Bleaching at different initial concentrations of CV (10–100 mg L^{-1}) was performed with bacterial biomass of 0.8 × 10^9 CFU mL^{-1} at 37 °C in the nutrient broth. The effect of bacterial concentration (0–3 × 10^9 CFU mL^{-1}) was evaluated with the CV concentration of 50 mg L^{-1} at 37 °C in the nutrient broth.

The bleaching rate was calculated at different incubation times using the formula:

$$\text{Bleaching}\,(\%) = \frac{\text{Abs}_0 - \text{Abs}_t}{\text{Abs}_0} \times 100, \quad (1)$$

where Abs_0 is the initial absorbance and Abs_t is the absorbance at the time t.

This formula is generally used to evaluate the dye decolorization for both biological processes (Ren et al. 2006a; Sun-Young et al. 2002) and plasma treatments (Merouani et al. 2013).

The bleaching experiments were performed in triplicate.

Glidarc plasma pre-treatment

The experimental apparatus is shown in Fig. 1, and has been described previously (Moussa et al. 2007; Djepang et al. 2014). Under operating conditions, an air compressor generated the feed gas. The air supplied (800 L h^{-1}) passes through a tube containing distilled water before being

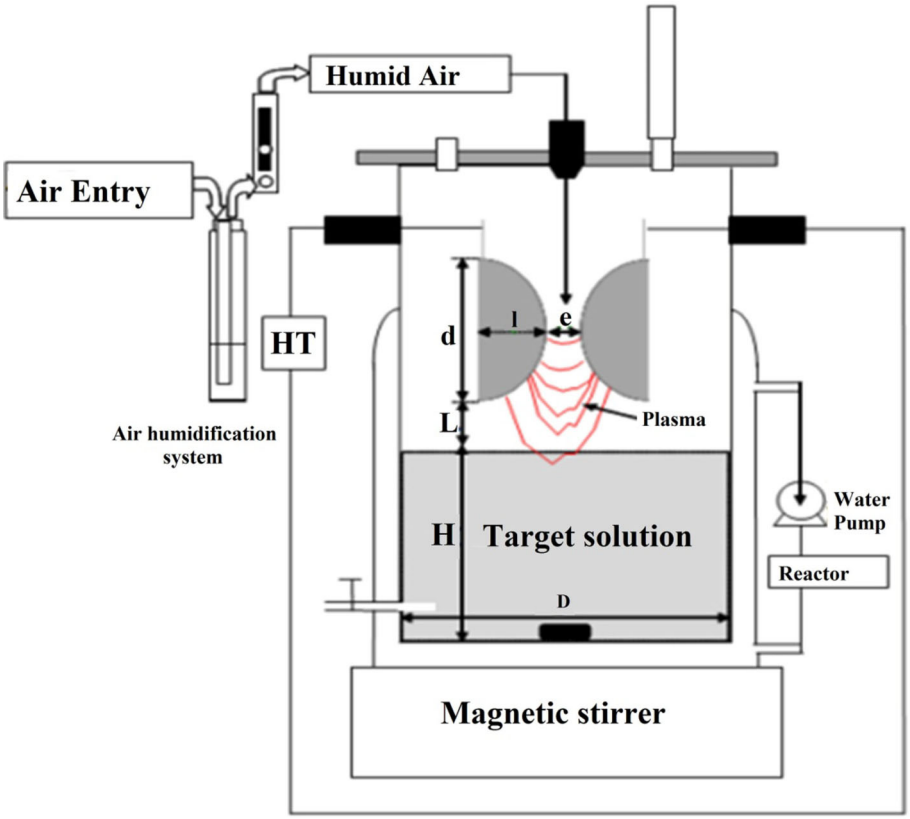

Fig. 1 Experimental device of glidarc plasma

injected along the electrodes through a nozzle. The nozzle diameter was 1 mm; the gap between the electrodes was $e = 2$ mm; the distance between the tips of the electrodes and the top of target solution was $L = 3$ cm; and the length of the electrodes was $d = 6$ cm. For each set of experiment, about 450 mL of CV (50 mg L^{-1}) solution was introduced into the reactor as target solution, stirred magnetically, and exposed to glidarc humid air plasma. The solution is thermostatted at 25 ± 2 °C by circulating water in a jacket. The plasma plume licks the surface of the target solution and allows chemical reactions to take place at the plasma solution interface. The treatment of the dye solution by the glidarc discharge was performed in the batch mode, and the residual concentration of CV for various exposure times t^* (5, 7, 10, 15, 30 min) was determined immediately after sampling with the spectrophotometer at $\lambda_{max} = 585$ nm, and bleaching rate was calculated according to Eq. (1).

The COD was determined by the potassium dichromate standard method, and the BOD_5 was evaluated according to OECD-301F test using the OXITOP system (manometric respirometry) (Clesceri et al. 2005). TOC was measured with Shimadzu TOC analyzer equipped with an autosampler (ASI-5000) and platinum-based catalyst. TOC analyzer was calibrated with a standard solution of potassium hydrogen phthalate. The PH was measured using pH meter HANNA HI 9811-5 fitted with a glass electrode.

For temporal post-discharge treatments, the CV solutions were initially exposed to the discharge for $t^* = 5$, 10, and 15 min, as described above. Once the discharge was switched off, the pre-treated solutions were left outside the reactor at the ambient temperature for various post-discharge times t_p (0–120 min), and then, the analyses were performed as in the case of direct exposure of CV solution to a continuous discharge.

Glidarc plasma/biological treatment coupling

The CV solution (50 mg L^{-1}) was pre-treated with plasma in continuous discharge, i.e., by exposing the dye directly to the plasma plume, for various times t^* (5, 10, and 15 min). The determination of the biodegradability index, BOD_5/COD and the COD/TOC ratio, was used to evaluate the biodegradability of plasma-pre-treated CV solution.

The plasma-pre-treated dye solution was used for the biological treatment with the *A. hydrophila* strain. Before this second step of coupling, the lethal effect of plasma (TPDR effects) was neutralized by adjusting the pH of the plasma-pre-treated solution at pH 6 with NaOH solution (Naïtali et al. 2010).

Results and discussion

Biodegradation of crystal violet by *Aeromonas hydrophila*

Effect of the state of bacterial cells on the bleaching

The effect of the state of bacterial cells on the bleaching of CV by *A. hydrophila* is shown in Fig. 2. Under static conditions at pH 7.5 and 37 °C, *A. hydrophila* (0.8×10^9 - CFU mL^{-1}) in stationary growth phase bleached 42 % of CV (50 mg L^{-1}) for 12-h incubation. The uninoculated sample showed no evidence of bleaching, indicating that the nutrient broth and the incubator have no effect on the decolorization. These biodegradation results are comparable to those obtained by Chimezie and Sawidis (2011) who reported that *A. hydrophila* removes 30 % of CV (50 mg L^{-1}) at 30 °C for 24 h of static incubation. *A. radiobacter* degraded 80 % of a less concentrated CV solution (10 mg L^{-1}) within 8 h (Parshetti et al. 2011), while *P. putida* decolorized 80 % of CV (25 mg L^{-1}) after 7 days (Chiing-Chang et al. 2007). To investigate the mechanism of microbial decolorization by *A. hydrophila*, the control test with heat-killed bacteria is useful. Indeed, this experiment helps to attribute the degradation to the adsorption of dye molecules onto microbial biomass and/or to the biodegradation of dye by the cells (Zhou and Zimmermann 1993; Sani and Banerjee 1999; Sun-Young et al. 2002; Ren et al. 2006a). In both cases, decolorization results from a change in the electron distribution in the dye molecule, either due to biological processes involving enzymes, or due to the formation of new bonds between the dye and the solid adsorbent in the case of adsorption (Saleh et al. 2014). In case of adsorption, the bacterial cells appear colored, but maintain their original color in case of biodegradation (Ren et al. 2006a). In this study, the bacterial cells were slightly colored; therefore, a part of degradation could result from adsorption. To confirm this hypothesis, a bleaching rate by only 16 % was obtained with heat-killed bacteria (Fig. 2), and this part was due to the adsorption. This bleaching rate corresponds to an increase in the pore surface area of the bacterial cells during autoclaving and then to the formation of new adsorption sites on the bacteria surface (Chen et al. 2003; Chimezie and Sawidis 2011; Ogugbue Chimezie et al. 2012). Hence, the bleaching rate resulting from adsorption caused by living bacteria in static condition should be very small.

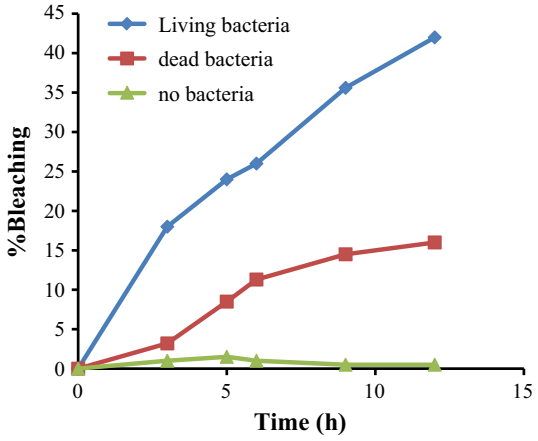

Fig. 2 Effect of the state of bacterial cells on bleaching, $T = 37$ °C, pH 7.5 (*A. hydrophila*) = 0.8×10^9 CFU mL^{-1}

However, a bleaching rate by 42 % was obtained with living cells, which implies that the mechanism can be attributed mainly to bacterial metabolism. As demonstrated in previous works, the biodegradation of TPM dye is caused by a soluble cytosolic enzyme, NADH/NADPH-dependent oxygenase secreted by *A. hydrophila* (Ren et al. 2006a, b; Ogugbue Chimezie et al. 2012), and is based on reduction and demethylation reactions (Parshetti et al. 2011). The color removal of CV (50 mg L^{-1}) by *A. hydrophila* in static condition, which is largely attributed to biodegradation, is relatively low (≤42 %) for 12-h incubation and needs to be increased.

Effect of bacterial concentration on the bleaching

Bleaching of CV solution was studied as a function of the bacterial concentration of inoculum (Fig. 3). The bleaching trends are similar regardless the initial bacterial concentration. Generally, for each incubation time, the percentage of bleaching of CV (50 mg L^{-1}) increased with the concentration of *A. hydrophila*. This is because the amount of enzyme secreted by bacteria increases with the cell concentration. Similar trends were obtained in previous works during the biodegradation of CV by *A. radiobacter* (Parshetti et al. 2011). After 9-h incubation, regardless of the bacterial

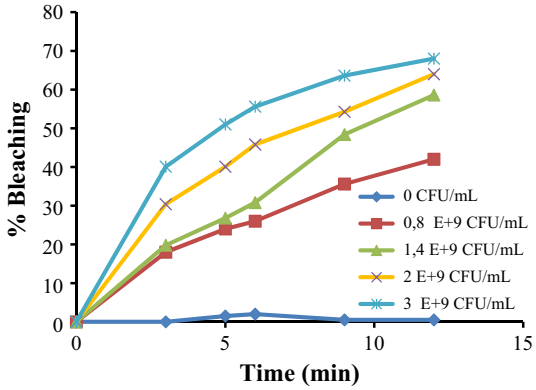

Fig. 3 Effect of bacterial concentration on bleaching of CV, $T = 37$ °C (CV) = 50 mg L^{-1}, pH 7.5

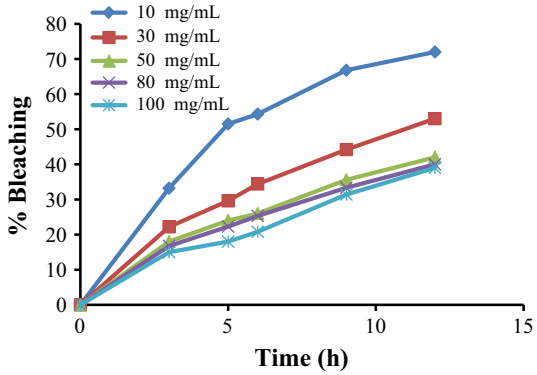

Fig. 4 Effect of initial concentration of CV on bleaching T = 37 °C (*A. hydrophila*) = 0.8×10^9 CFU mL^{-1}, pH 7.5

concentration, the bleaching rate increases slightly (Fig. 3) because of the low nutrient content of the medium.

The kinetic studies showed that the biodegradation of CV by *A. hydrophila* followed pseudo-first-order kinetics regardless of the initial bacterial concentration (not shown here). For example, with an initial bacterial concentration of 0.8×10^9 CFU mL^{-1} and dye concentration of 50 mg L^{-1}, the plot of $\ln(Abs_0/Abs_t)$ versus time (h) presented a linear plot with an average rate constant $k_b = 0.044\ h^{-1} = 7.33 \times 10^{-4}\ min^{-1}$ (the relevant coefficient $R^2 = 0.985$). The rate constant k_b (h^{-1}) increased with the initial bacterial concentration N_0 (CFU mL^{-1}) according to a reasonably linear relationship:

$$k_b = 2.67 \times 10^{-11} N_0 + 0.0286 \quad (R^2 = 0.94) \qquad (2)$$

This finding confirms that the biodegradation of organic compounds at low concentrations generally follows first-order kinetics (Feng et al. 2004; Siciliano and De Rosa 2015), which corroborates Durai et al.'s (2011) study of the biodegradation of tannery effluents in a batch bioreactor.

Although the bleaching rate could be enhanced by increasing the initial bacterial concentration, or even by considering the highest concentration used, the bleaching remains incomplete.

Effect of pollutant concentration on bleaching

The influence of the initial CV concentration C_0 on the biodegradation rate was also considered. With the same bacterial biomass (0.8×10^9 CFU mL^{-1}), bleaching rates of CV decrease with increasing C_0: Bleaching rates of 72 and 42 % were obtained for 10 and 50 mg L^{-1} of CV, respectively, for 12-h incubation (Fig. 4). However, between 50 and 100 mg L^{-1} of CV, no significant change in the bleaching rate was observed. Rajesh and Uttam (1999) have also shown that the CV decolorization by *Kurthia* sp. decreased as the concentration of the dye increased. Above 50 mg L^{-1} of CV, the metabolism of *A. hydrophila* cells was inhibited probably because the cells are in the stationary phase. On the contrary, other bacteria such as *Citrobacter* sp. and *B. cereus*, in the growth phase, have a high bleaching rate even with high concentrations of organic pollutants (Sun-Young et al. 2002; Yousefi Kebria et al. 2009).

Biodegradability enhancement of CV by glidarc plasma

Bleaching and degradation of CV by direct exposure to a continuous discharge

Direct exposure of the CV solution to the glidarc discharge for time t^* (i.e., continuous discharge) was investigated previously by Abdelmalek et al. (2006) who was a pioneer in the plasma bleaching/degradation of dye-stuff. Figure 5 shows that the initial concentration of CV solution decreased during the plasma treatment. The initial pH of 5.4 decreases during the first minutes of treatment and remains around 3.5 throughout the treatment (not shown here), as observed in many earlier studies (Moussa et al. 2007; Doubla et al. 2008; Njoyim-Tamungang et al. 2009; Djepang et al. 2014). The degradation of the dye was followed by the determination of COD, and the bleaching was followed by residual concentration (absorbance). Figure 5 presents the evolution of these parameters. Bleaching and degradation occur simultaneously from the first few minutes of treatment. The bleaching rate was higher than the degradation rate especially during the first few minutes. For 5-min treatment, COD abatement was 13 %, while decolorization abatement was higher (two times the COD abatement), i.e., 28.5 %. For $t^* = 30$ min, 67 % of

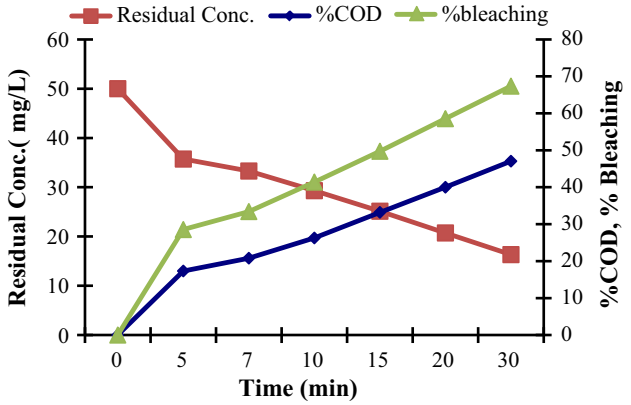

Fig. 5 Evolution of residual concentration, bleaching, and COD removal of CV during direct exposure of CV to discharge

bleaching and 35 % of degradation were obtained (Fig. 5). This difference can be explained by the fact that bleaching results from the combined effects of acidification by plasma (due to the formation of transient nitrous acid and nitric acid) and oxidation by oxidizing agents such as primary species $HO^{\cdot}$ ($E^{\cdot}_{(^{\cdot}OH/H_2O)} = 2.8\,V/SHE$), and $NO^{\cdot}$ ($E^{\cdot}_{(NO^{+}/NO^{\cdot})} = 1.21\,V/SHE$). Bleaching results from derivatives species: H_2O_2 ($E^{\cdot}_{(H_2O_2/H_2O)} = 1.8\,V/SHE$), peroxynitrous acid ONOOH ($E^{\cdot}_{(ONOOH/NO_2)} = 2.02\,V/SHE$), and peroxynitrites $ONOO^{-}$ ($E^{\cdot}_{(^{\cdot}NO/ONOO-)} = 2.1\,V/SHE$); while degradation is only caused by these oxidizing agents (Doubla et al. 2008; Iya-Sou et al. 2011; Merouani et al. 2013). These trends are similar to those obtained by Abdelmalek et al. (2006). Plasma decolorization is due to the cleavage of the –C=C– and –N=C– chromophore groups of the CV molecule, while degradation requires the cleavage of chromophore groups but also the opening of the aromatic rings and grafting of nitrogen containing groups provided by $ONOO^{-}$ (Brisset et al. 2008; Naïtali et al. 2012).

The bleaching and the degradation kinetics followed pseudo-first-order kinetics, obtained by the linear plots of the absorbance ln A_{585} and ln COD versus t^* (not shown). The calculated rate constants were $k_{ble} = 4.2 \times 10^{-2}$ - min^{-1} ($R^2 = 0.984$) and $k_{deg} = 1.4 \times 10^{-2}\ min^{-1}$ ($R^2 = 0.964$) for bleaching and degradation, respectively.

Bleaching of crystal violet in post-discharge conditions

CV samples were exposed to the gliding electric discharge for various times t^* (5, 10, and 15 min). After each exposure, the samples were disposed outside the plasma reactor and abandoned for times t_p (30, 60, 90, and 120 min) in post-discharge conditions, i.e., in the absence of any extra energy source. The color removal of CV increased with post-discharge time. For exposure time $t^* = 5$ min, the color abatement was 28.6 % immediately after switching off the discharge ($t_p = 0$ min); the rates were 32.5, 34.5, 37, and 39 % for t_p: 30, 60, 90, and 120 min post-discharge, respectively (Fig. 6). The bleaching reaction during post-discharge also followed pseudo-first-order kinetics with an average rate constant of $1.67 \times 10^{-3}\ min^{-1}$ for $t^* = 5$ min. The results presented in Fig. 6 confirm the occurrence of TPDR for the other exposure times t^*(10 and 15 min).

Generally, the authors attribute the degradation in TPDR conditions to the species H_2O_2 and HNO_2 formed in the discharge (Kamgang-Youbi et al. 2007; Moussa et al. 2007). The authors also consider the great influence of peroxynitrites $ONOO^{-}$ and its matching acid, which are soluble in water; able to dissociate into $^{\cdot}OH$ and ONO^{-}; and to induce oxidizing, nitrating, and nitrosing post-discharge effects on solutes (Naïtali et al. 2012; Merouani et al. 2013).

It is important to know that the TPDR active species are pH-dependent. Indeed, when the pH of the dye solution was not adjusted immediately after exposure (pH around 3.5), the residual concentration of the target liquid decreased during post-discharge, meaning that bleaching and degradation evolve, as presented above. On the contrary, when the pH was adjusted to 6, the concentration of CV remained constant during the post-discharge (Fig. 6). So, the pH can be used to neutralize reactive species responsible for the post-discharge phenomenon. This

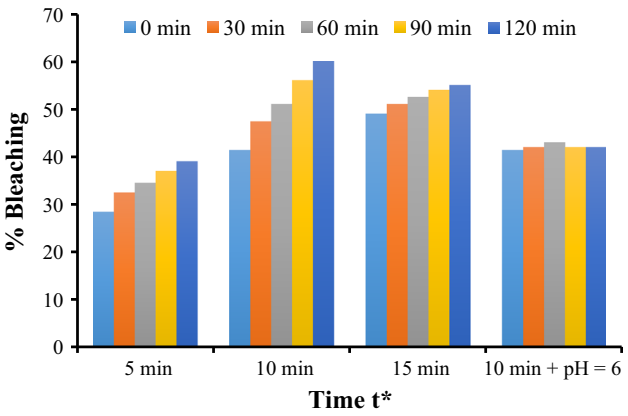

Fig. 6 Evolution of bleaching percentage and pH effect on reactivity of species ($t^* = 10$ min) during post-discharge treatment of CV ($t_p = 0$–120 min)

method was used by Naïtali et al. (2010) to stop the lethal effect of the glidarc plasma on the microorganisms in TPDR conditions and can be used in this work in order to demonstrate the effectiveness of the second step of coupling.

Effect of plasma on the biodegradability

The evolution of COD, BOD_5, and TOC during direct exposure of CV solution to the discharge is shown in Fig. 7. COD decreases from 170 to 110 mg L^{-1}, and BOD_5 increases from 20 to 40 mg L^{-1} for 30 min of glidarc treatment, meaning that there is a conversion of the COD to BOD_5. Marco et al. (1997) reported that the treatment of organic compounds such as 2,4-diclorophenol by an AOP, ozonation, increases BOD_5 from 0 to 55 mg L^{-1} after 60 min of treatment. A decrease in TOC from 41 to 32.5 mg L^{-1} was observed for 30 min of treatment with a TOC abatement of 20.7 % indicating a low mineralization of CV. Abdelmalek et al. (2008) achieved only 19 % of TOC abatement for 30-min degradation of bisphenol A by glidarc plasma. Therefore, the by-products of CV degradation for 30 min of glidarc treatment are still organic compounds.

The evolution of the COD/TOC ratio and the biodegradability index DBO_5/COD during the glidarc treatment of CV is shown in Fig. 8. The COD/TOC ratio provides information on the oxidation state of organic substances in solution (Marco et al. 1997). For alkanes, this parameter is between 4 and 5.3, while for strong oxidants such as oxalic acid, the value is 0.6 (Marco et al. 1997). During the plasma treatment, the COD/TOC ratio decreased, from 4.15 for the initial solution to 3.38 for

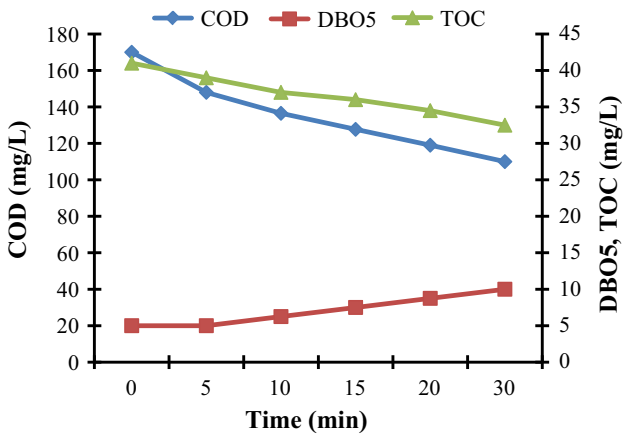

Fig. 7 Evolution of COD, DBO_5, TOC during plasma treatment of CV in continuous discharge

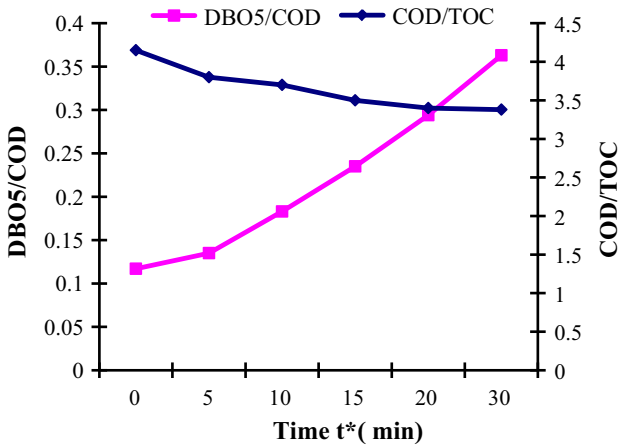

Fig. 8 Evolution of biodegradability index and COD/TOC ratio of CV during plasma treatment

$t^* = 30$ min (Fig. 8). This decrease is due to the high oxidation degree of organic substances in solution. The biodegradability index DBO_5/COD increased significantly during treatment, rising from 0.11 for the initial solution of CV, to 0.36 for 30-min treatment. Given that only the effluents with DBO_5/COD ratio $\geq$0.33 are considered as biodegradable (Goi et al. 2004; García-Montaño et al. 2006), the 30-min plasma-treated CV is therefore biodegradable. For $t^* < 30$ min, the resulting effluents were not biodegradable. Indeed, for $t^* = 5$, 10, 15, and 20 min, the biodegradability indexes were 0.13, 0.18, 0.23, and 0.29, respectively. Although these values were <0.33 (for $t^* < 30$ min), one can note that there is still an increase in biodegradability. Previous works also demonstrated that AOPs (ozonation, photocatalysis …) increase biodegradability of non-biodegradable organic pollutants (Mantzavinos and Psillakis 2004; Farre et al. 2005; Zapata et al. 2010). Since mineralization was low (Fig. 7), the by-products formed for $t^* \leq 30$ min during plasma-chemical oxidation are biodegradable organic compounds or some species which are less biorecalcitrant than CV. Among the factors that contribute to the biodegradability enhancement, we can mention the partial mineralization of the pollutant molecule, the conversion of the aromatic to aliphatic compounds by ring-opening, ring-hydroxylation and detoxification (Goi et al. 2004; Khenniche et al. 2015). During the plasma treatment, strong oxidizing agents such as $OH^{\cdot}$ and $NO^{\cdot}$ (and derivatives) can attack at the aromatic rings causing ring-opening, or at the carbon in α-position with respect to electrophilic central carbon of CV to yield the cleavage of conjugated chromophores structures. Carboxylic acids, N-aminobenzene, which are more biodegradable than the CV could be obtained inter alia

(Huan-Jung et al. 2009). The aim of this section was not to demonstrate the reaction mechanism of degradation, but to assert merely that the degradation was due to glidarc oxidizing agents. Previous works reported that the N-demethylation and cleavage of conjugated chromophore structures are plausible mechanisms of degradation of CV by Fenton processes (Huan-Jung et al. 2009). These authors have identified inter alia pararosaniline and the 4,4′-bis-aminobenzophenone as by-products of CV degradation. Given that it is not necessary to pre-treat the CV for long durations, the plasma pre-treatment times of 5, 10, and 15 min could be used to study the glidarc plasma/biodegradation coupling process.

Plasma-chemical oxidation/biodegradation coupling

Glidarc direct exposure followed by biodegradation

The evolution of the bleaching rate during the biodegradation by *A. hydrophila* (0.8×10^9 CFU mL^{-1}) of the CV pre-treated for various times t*with glidarc plasma is shown in Fig. 9. For 5-, 10-, and 15-min plasma treatment alone, the bleaching rates were 28.5, 41.4, and 49.7 %, respectively (0-h incubation). After glidarc pre-treatment, the glidarc long life species were first neutralized with NaOH solution, and then, the biological treatment by *A. hydrophila* was carried out under the same conditions with no pre-treated samples (Fig. 2). For all pre-treated samples, the bleaching rates were higher than those obtained with the no pre-treated samples ($t^* = 0$ min). For example, the bleaching rates for 5-min plasma-pre-treated solution were 28.5, 60, 65, and 70 % for 0-, 2-, 4-, and 6-h incubation, respectively, compared to the sample not pre-treated ($t^* = 0$ min), with bleaching rates 0, 12, 22, and 26 % for 0, 2, 4, and 6 h of incubation, respectively (Fig. 9); there is an enhancement of biodegradation by glidarc. This enhancement was also evidenced for the other pre-treated samples (10 and 15 min). For all pre-treated samples, the bleaching rate increased with the incubation time same as the samples directly treated by *A. hydrophila* without glidarc exposure. One also observed the influence of the glidarc exposure duration on bleaching rates during the biodegradation of plasma-pre-treated CV: For 2-h incubation, the values of the bleaching rate were enhanced by 31.5, 42.6, and 42.3 %, respectively, for 5, 10, and 15 min of glidarc exposure. For 5, 10, and 15 min of plasma-chemical oxidation, the color abatements by 70, 95, and 100 %, respectively, were obtained for 6-h biodegradation, and the bleaching rate enhancements were 41.5, 53.6, and 50.3 %, respectively.

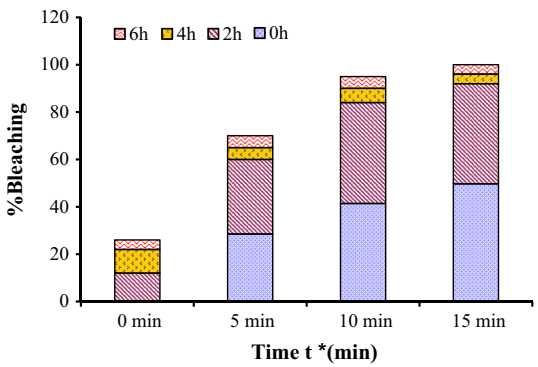

Fig. 9 Evolution of bleaching during biodegradation of CV pre-treated with plasma at different time t^* (*A. hydrophila*) $= 0.8 \times 10^9$ - CFU mL^{-1}, $T = 37$ °C, pH 7.5

The high color abatement values could be explained by the cumulative effects of plasma-pre-treatment and biodegradation, and by the formation of more biodegradable by-products: for example, $DBO_5/COD = 0.23$ for $t^* = 15$ min higher than 0.11 for $t^* = 0$ min.

The color removal of CV solution was not significant enough (70 %) at the end of plasma glidarc/biodegradation coupling of the 5-min pre-treated sample: This is due to the poor biodegradability of CV solution after 5 min of plasma-chemical oxidation ($DBO_5/COD = 0.13$), given that there is a small amount of biodegradable by-products in solution. García-Montaño et al. (2006) achieved 100 % of color abatement for a long biological treatment time (48 h) during the removal of hetero-bireactive dye by the combination of photo-Fenton process and aerobic sequencing batch reactor.

AOPs generally enhance a biodegradation process, because AOPs are able to convert recalcitrant compounds to biodegradable by-products. The degradation of malachite green (TPM dye) by the electro-Fenton process yields biodegradable by-products (Oturan et al. 2008). The results of this study corroborate those of Ballesteros et al. (2009) on pesticides degradation by photo-Fenton oxidation/biodegradation coupling. These authors demonstrated that pesticides mineralized at 31 % by photocatalytic pre-treatment could be completely mineralized for 5-h biological treatment.

Comparison of glidarc plasma/biodegradation coupling with post-discharge

The bleaching rates of CV treated by the different methods used in this study are presented in Fig. 10. For 5, 10, and 15 min of direct exposure of CV to glidarc, the bleaching rates were 28.5, 41.4, and 49.7 %, respectively. For these exposure times t^* (5, 10, and 15 min), 2-h TPDR led to abatements of 39, 60, and 55 %, respectively, whereas 2-h biological treatment led to 60, 84, and 92 % bleaching rates, respectively, after 5-, 10-, and 15-min glidarc exposure. As already mentioned, temporal post-discharge increases the bleaching rates, but the results obtained in

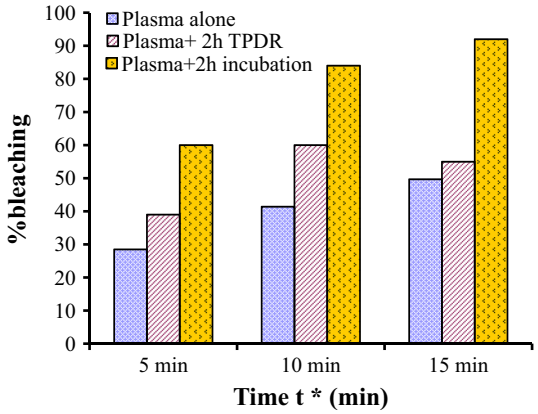

Fig. 10 Comparison of glidarc plasma/biodegradation coupling and temporal post-discharge treatment of CV (*A. hydrophila*) = 0.8 × 10^9 CFU mL^{-1}, $T = 37$ °C, pH 7.5

this study evidence a better enhancement when the biological process was coupled with glidarc pre-treatment. These results justify the use and the efficiency of glidarc plasma/biodegradation coupling for the treatment of textile wastewater.

Conclusion

This paper reports on the ability of gliding arc discharge to enhance the biodegradation of a TPM dye by *A. hydrophila* strain. The results showed that *A. hydrophila* strain in the stationary growth phase bleaches 42 % of CV (50 mg L^{-1}) for 12-h incubation at 37 °C. Biodegradation of CV by *A. hydrophila* follows pseudo-first-order kinetics. Bleaching and degradation of CV by plasma glidarc obey a pseudo-first-order kinetic with rate constants of 4.2 × 10^{-2} and 1.4 × 10^{-2} min^{-1}, respectively. For 30-min exposure to the plasma, 67 % of color removal was obtained, while COD abatement was 35 %. Post-discharge kinetics abatement also followed pseudo-first-order kinetics. The results have shown that 15 min of glidarc plasma treatment is the optimal pre-treatment time: Biodegradability index DBO_5/COD of water polluted by the CV increases from 0.11 to 0.23, while mineralization was only by 12.2 %. For this time, bleaching rate was 49.7 %, and after 2 h in TPDR, this rate increased to 55 %, and when the biological process was coupled, the bleaching rate was 92 % for 2-h incubation and 100 % bleaching was achieved for 6-h incubation. The glidarc plasma/biodegradation coupling increases the efficiency of the wastewater treatment and reduces the biodegradation time. There is a positive synergism of bacterial and plasma treatments. This coupling also reduces energy cost associated with complete mineralization of wastewater by nonthermal plasma processes.

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