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Direct yellow degradation by combined Fenton-enzymatic process

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Abstract

The removal of direct yellow 106 (DY106, C.I.40300) by a combined process of Fenton oxidation and enzymatic degradation in a sequential or mixed batch reactor is discussed. Experiments were first conducted with the enzymatic and chemical oxidation processes separately in order to determine the effects of various parameters such as pH, ferrous ion, hydrogen peroxide, dye and enzyme concentrations on the overall yield and kinetics of both processes. Decolourization was followed by UV-visible spectroscopy and high performance liquid chromatography (HPLC). Results showed that 89.5 % of DY106 were removed by enzymatic treatment after only 2 min, while 10 min of contact time were necessary to eliminate more than 98 % of 50 mg/L by Fenton's process. A high performance was achieved under optimized conditions by the mixed combined process with time reduction down to 5 min. The study was also conducted to evaluate the efficiency of combined Fenton's reaction as a pre-treatment and post treatment process combined with C-peroxidase at different ferrous ions concentrations. The optimal doses of Fe^{2+} were 2.5 mM, 1m M and 0.8 mM for Fenton, Fenton-enzymatic sequential and mixed processes. Intermediate products absorbing in UV range were detected for single Fenton or enzymatic treatment but were eliminated in all combined enzymatic-Fenton processes. Phytotoxicity tests showed that no toxicity was detected after treatment by combined process.

Introduction

Pollution of water by dyes is a serious problem in the different countries. Many industries use dyes to colour their products. Furthermore, the expanded use of dyes has shown that some of them and some of their reaction products, such as aromatic amines, are highly carcinogenic (Banat *et al.* 1996; Robinson *et al.* 2001). The removal of dyes (colour) from wastewater is, therefore, a challenge to the related industries. Due to the variability of the organic dyes and the resultant waste solution, wastewater containing dyes is difficult to treat using classical methods. These methods have lot of disadvantages such as transfer of pollutants from one phase to another; some of them are non-destructive, costly methods and could generate secondary waste products such as sludge which may need further disposal (El-Desoky *et al.* 2010; Lu and Liu 2010; Jonstrup *et al.* 2011; Chhabra *et al.* 2015). Among the chemical processes, Fenton's oxidation is one of the earliest Advanced Oxidation Processes (AOP) that was successfully used in dye degradation. The simplicity and relative

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low cost of the process made it a promising and attractive treatment method for the effective decolourization and degradation of dyes (Arslan *et al.* 2000; Meric *et al.* 2004).

In Fenton system, ferrous ions react with hydrogen peroxide at acidic pH producing hydroxyl radicals ($HO^{\bullet}$) with powerful oxidizing abilities to degrade organic pollutants (Neamtu *et al.* 2003; El-Desoky *et al.* 2010) (Eq. 1):

$$H_2O_2 + Fe^{2+} \rightarrow HO^{\bullet} + OH^- + Fe^{3+} \quad (1)$$

Hydroxyl radicals may react with ferrous ions to form ferric ions or react with organics (Eq. 2, 3):

$$HO^{\bullet} + Fe^{2+} \rightarrow OH^- + Fe^{3+} \quad (2)$$

$$HO^{\bullet} + \text{Organics} \rightarrow \text{Products} \quad (3)$$

However, at high pH values in the Fenton process (pH > 4) ferric ions precipitate onto various iron hydroxyls, this leads to production of sludge containing high amount of Fe (III), which need to be treated by safe disposal methods. Other kind of problems could occur, such as the generation of aromatic amines and high reagent costs (Neamtu *et al* 2003; Lu and Liu, 2010).

Plant peroxidases are potential catalysts that could be used in the degradation and/or elimination of aromatic pollutants (Mohan *et al* 2005). These enzymes can catalyze the transformation of organic pollutants present at very low concentrations (Akhtar and Husain 2006). Peroxidases from gourd or bitter gourd are highly effective in decolourizing wide spectrum of industrially important azo dyes (Akhtar *et al.* 2005; Boucherit *et al.* 2013). Maximum decolourization efficiency is obtained with a limited amount of enzyme under mild conditions (Schmitt *et al.* 2012). Further, in certain cases enzyme can lead to forming toxic aromatic amines (Ali *et al.* 2013). From the above discussion it seems that use of either Fenton or enzymatic process could generate some drawbacks.

Combined chemical-biological processes were also carried out by many researchers for treating recalcitrant coloured compounds. Thus, the coloured solutions were treated in a sequential or mixed batch with a chemical oxidation to partially degrade recalcitrant organic compounds. This pretreatment leads to a discoloured solution, which is readily biodegradable by bacteria or enzymes (Karimi *et al.* 2009; Mandal *et al.* 2010; Oller *et al.* 2011). Karimi *et al.* (2009) showed that sequential coupling of enzymatic and photo-Fenton processes were promising solutions for decolourization of pulping waste water. Similarly, Mandal *et al.* (2010) achieved total decoulourization by sequential Fenton-biological process. Nevertheless, such results were obtained under high hydrogen peroxide (111 g/L) and iron sulfate (6 g/L) concentrations. The duration of the process (3 days) was also an inconvenient. Besides, some dyes with complex structures could inhibit microbial growth and their biodegradation by-products could be more toxic. On the other hand, the advanced oxidation process by Fenton's reagents is not cost effective while the biological treatment by micro-organisms is too much time consuming (Lucas *et al.* 2007). It is therefore quite interesting to explore the feasibility of a hybrid, Fenton-enzymatic process, in the treatment of dye pollution as an alternative to limited individual treatments.

In this context we studied the effectiveness of the combined sequential or mixed Fenton-enzyme process under optimal conditions of individual processes. Attention was focused on the effects of iron concentration and contact time on combined process efficiency comparatively with individual processes. The proposed strategy aims to obtaining the highest qualitative and quantitative efficiency by reducing the process duration and reactants concentrations.

Experimental

Dye

Direct azo dye investigated: C.I. Direct yellow 106 (DY106, C.I. 40300), was provided by SOITEX (textile manufacturing unit in Tlemcen (Algeria), and which was purchased from Ciba Colours Ltd. UV-visible region was selected at 396 nm. The structure of dye is showed in Fig. 1.

Reagents for enzymatic treatment

Cucurbita pepo (courgette) peroxidase (C-Peroxidase) was extracted from fresh Courgette fruit, of local market. Acetone, buffers solutions, hydrogen peroxide (30 % v/v), were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Others

chemicals were of analytical grade and were used without further purification.

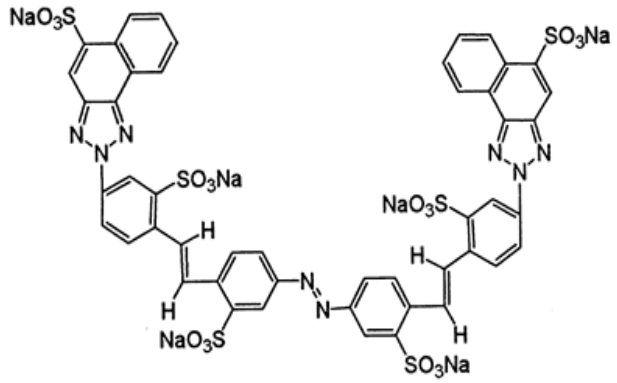

Fig. 1. Chemical structure of DY106.

Reagents for Fenton treatment

Hydrogen peroxide (30 %, *v/v*), hydrochloric acid (37 %), sodium hydroxide, and Fe^{2+} ion as $FeSO_4.7H_2O$, all were purchased from Sigma-Aldrich Corporation. (St. Louis, MO, USA).

Enzymatic treatment

Enzymatic assay

Activity of C-peroxidase was assessed by colorimetric estimation using phenol and hydrogen peroxide as substrates and 4-aminoantipyrene as a chromogen (Nicell and Wright 1997).

Treatment conditions

Batch experiments were conducted in a glassy reactor with a working volume of 0.l L. Reactor contents were kept under magnetic stirring. These experiments were carried out under the following conditions: initial dye concentration (varying from 10 to 110 mg/L), hydrogen peroxide concentration (0.1 – 7.5 mM), enzymatic activity (0.16 – 3 IU/mL) and pH (2.0 – 10.0). The effect of pH on decolourization was investigated by performing different type of buffers including: 20 mM potassium chloride/HCl buffer (pH 2), 20 mM potassium phthalate/HCl buffer (pH 3, 4), 20 mM potassium phthalate/NaOH buffer (pH 5), 20 mM potassium phosphate/NaOH buffer (pH 6, 7, 8), 17.5 mM borax/NaOH buffer (pH 9, 10).

Fenton treatment

The effects of pH, concentrations of hydrogen peroxide, ferrous ions and dye were investigated in order to determine the optimal conditions for the oxidation of organic dye.
All Fenton experiments were run at 24 °C in a working volume of 0.1 L containing 50 mg/L of dye solution with initial pH in range (2 to 8). In order to initiate Fenton reaction, 8 mM of H_2O_2 and 1 mM of Fe^{+2} were added to reaction mixture. Magnetic stirrer was used to provide continuous mixing during Fenton experiments. The reaction was immediately stopped after 15 min by pH adjustment to 10. The mixture was centrifuged at 4,000 rpm for 10 min. Quantitative estimation of the residual dye concentration after treatment with Fenton was carried out on the supernatant at the maximum wavelength 396 nm. All experiments were conducted in duplicate and their average values were reported.

Enzymatic-Fenton treatment

After optimization of enzymatic and Fenton treatments separately, different configurations (for fixed dye concentration of 50 mg/L) of combined enzymatic-Fenton treatment were conducted as follows: sequential batch of enzymatic-Fenton, or Fenton-enzymatic processes and mixed batch. The objective was to determine the best combination which gives the highest yield, the shortest time duration and minimum residual by-products for minimum ferrous dose. All combined treatment experiments were conducted in glass reactors containing 0.1 L of reaction mixture.

Sequential Batch

Fenton-Enzyme sequential batch

In the first stage, aqueous dye solution was treated by Fenton treatment (FT). Initial conditions were: 50 mg/L of dye concentration, 2.5 mM of Fe^{2+} and 20 mM potassium chloride/HCl buffer (pH 2). The reaction was started by addition of 8 mM of H_2O_2. After 15min of Fenton's treatment, the mixture was centrifuged after neutralisation with NaOH (1 M). The Enzymatic reaction was started after pH adjustment to 2 by addition of 1.7 IU/mL

of C-peroxidase. The treated samples were neutralized to a pH of 10, by ammonium liquor after 10 min. The mixture was centrifuged and the supernatant was analysed by UV-visible spectrophotometer.

Enzyme-Fenton sequential batch

The reaction mixture contained initially 50 mg/L of dye, 1.7 IU/mL of C-peroxidase in 20 mM potassium chloride/HCl buffer (pH 2). The reaction was started by addition of 1 mM of H_2O_2. At the end of the enzymatic treatment (2 min), the mixture was centrifuged. The supernatant was then treated with Fenton process by addition of Fe^{+2} (2.5 mM) and H_2O_2 (7 mM). At the end of the process (10 min), the pH was adjusted to 10 by adding ammonium liquor and centrifuged. The supernatant was analysed by UV-visible spectrophotometer.

Mixed batch

For the mixed batch treatment, a volume of 100 mL was prepared as follows: 50 mg/L of DY106, and enzymatic activity 1.7 IU/mL of C-peroxidase, 50 mM of potassium chloride/HCl buffer (pH 2) and 2.5 mM of Fe^{2+}. The reaction was started by addition of 8 mM of H_2O_2. After 30 min, the reaction was stopped by adjusting pH to 10 with ammonium liquor. The mixture was centrifuged and the supernatant separated and analyzed by UV-visible spectrophotometer.
In all cases of combined process, Fe^{2+} concentration was optimized in order to reach a maximum yield of decolourization. For mixed process, kinetics was followed by withdrawal of aliquots of 5 mL of treated dye solution at different time intervals.
The decolourization efficiency (DE) was expressed as the percentage ratio of the decolourized dye concentration to that of initial one and was calculated as follows (Eq. 4):

$$DE(\%) = \frac{A_0 - A_t}{A_t} \quad (4)$$

where A_0 – initial dye absorbance at 396 nm; A_t – dye absorbance at time t at 396 nm.
For enzymatic process, the initial decolourization rate (IDR) was calculated from the slope of the dye concentration versus time curve for different initial dye concentration (results not shown).
The optimal concentration of 50 mg/L was obtained through a one factor at a time experimental strategy, by choosing initial reaction rate and degradation yield as responses, for both enzymatic and Fenton process. Then, the same concentration was chosen for the combined processes.

Analytical methods

Dye decolourization was measured by monitoring the absorbance with a UV-Visible spectrophotometer (Model Perkin-Elmer 550 A) based on the maximum absorbance. The maximum absorbance was obtained by preparing a solution in a selected buffer with a known concentration and measuring the absorbance by UV-visible at different wavelengths from 190 to 700 nm.
UV-visible spectral analysis (between 200 to 500 nm) was also carried out in order to evaluate changes in absorption spectrum before and after treatment and, eventually, detect the appearance or disappearance of any by-products.
High performance liquid chromatography (HPLC) was carried out on a Shimadzu equipped with a UV detector (SPD10A) fixed at 254 nm. Samples were analyzed on a C18 column. An isocratic method with the 0.025 M phosphate buffer (pH 3.0): acetonitrile mobile phase was employed in the separation (40 : 60) (*v/v*). The injection volume was 20 µL. The flow rate was kept at 1 mL/min during the run under 25 °C.

Phytotoxicity studies

Phytotoxicity tests were conducted to assess the impact of the treated and untreated coloured water on growth of plants. This method was developed to ascertain the toxicity of polluted liquid samples on seed germination and root elongation as an indication of the possibility to use it in irrigation or recycling for industrial or domestic purposes (Di Salvatore *et al.* 2008). The ethyl acetate extracted products of DY106 degradation obtained from different treatments and the original dye were dissolved in sterile distilled water to make a final concentration of 100 ppm. The experiments were carried out on common

beans (*Phaseolus vulgaris* L.), a fast growing and sensitive plant. Ten seeds of plant were sowed into a plastic sand pot. The sand pot was prepared by adding 20 g washed sand into the plastic pot. Then, each pot was irrigated with 5 mL (100 ppm) of each solution per day. Control set was executed using distilled water at the same time. Germination rate (%), length of plumule (shoot) and radicle (root) were recorded after 7 days (Parshetti *et al.* 2006).

Results and Discussion

Enzymatic treatment, optimal pH, dye, H_2O_2, enzyme concentrations and time duration

The variation of dye removal (for initial concentration of 50 mg/L) by free peroxidase at various pH values is depicted in Fig. 2. About 73.7 % of dye was removed at pH 2 under the specified experimental conditions of dye (50 mg/L), H_2O_2 (1 mM) and enzymatic activity (1.4 IU/mL). The efficiency of enzymatic dye removal decreased for higher pH values between 3 and 10. Similar results were established with other plant peroxidases for the treatment of different azo dyes. Maximum degradation was observed within the pH range of 2 to 5 (Mohan *et al.* 2005; Kalsoom *et al.* 2015). The concentration of the dye has significant influence on enzyme-mediated reaction. Enzymatic reactions are significantly affected by concentration of substrate (Fig. 3). If the amount of substrate increases gradually for a constant enzyme concentration, the rate of reaction will increase to reach a maximum constant value (more than 9 mg/min.L). However, the decolourization efficiency decreased for the same change of dye concentration. Similar results were obtained with different type of dyes by plant peroxidases within the concentration range of 20 – 50 mg/L (Mohan *et al.* 2005; Bhatti *et al.* 2012; Ahmedi *et al.*, (2012). Maximum decolourization of solar blue A and solar flavine 5G has been reported with dye dose of 20 mg/L for a peroxidase activity of 12 IU/mL, H_2O_2 dose of 0.8 and 0.7 mM, respectively, at pH 4 (Bhatti *et al.* 2012). Also Ahmedi *et al.* (2012) showed that optimal concentration of Congo red giving maximum decolourization by turnip peroxidase is 50 mg/L for a dose H_2O_2 of 50 mM, peroxidase activity of 0.45 IU/mL at pH 2. Mohan *et al.* (2005) obtained maximum decolourization of acid black 10 BX with enzyme activity of HRP of 2.94 IU/mL. The optimal dye concentration of 50 mg/L was selected as the intersection point between initial rate and efficiency of decolourization curves.

In order to find out optimal H_2O_2 concentration, experiments were carried out by varying peroxide concentration and keeping other experiment conditions unchanged. Hydrogen peroxide was considered as a co-substrate. It contributes in the catalytic cycle of peroxidase, to oxidize the native enzyme into a reactive intermediate radical, which accepts the aromatic substrates and converts them

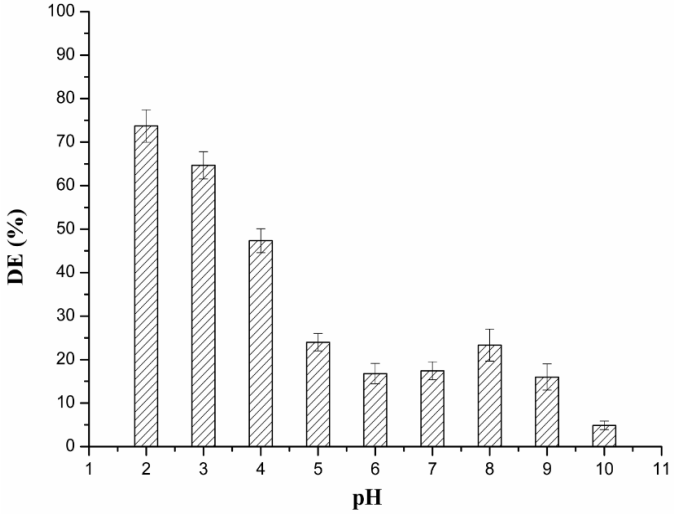

Fig. 2. Effect of pH on dye decolourization by C-peroxidase. (T = 24 °C, 50 mg/L of DY106, 1 mM of H_2O_2 and 1.4 IU/mL of C-peroxidase).

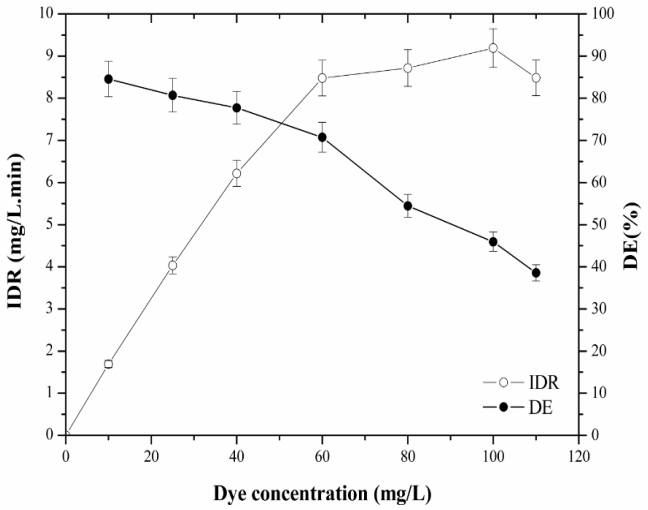

Fig. 3. Effect of initial dye concentration on IDR and DE by C-peroxidase. (T = 24 °C, 1 mM of H_2O_2 and 1.4 IU/mL of C-peroxidase).

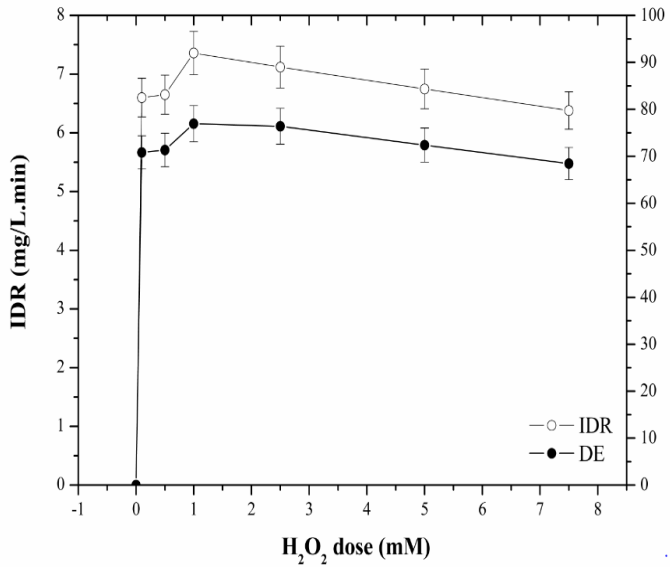

Fig. 4. Effect of H_2O_2 dose on rate and efficiency of decolourization by C-peroxidase. (pH 2, T = 24 °C, 50 mg/L of DY106, and 1.4 IU/mL of C-peroxidase).

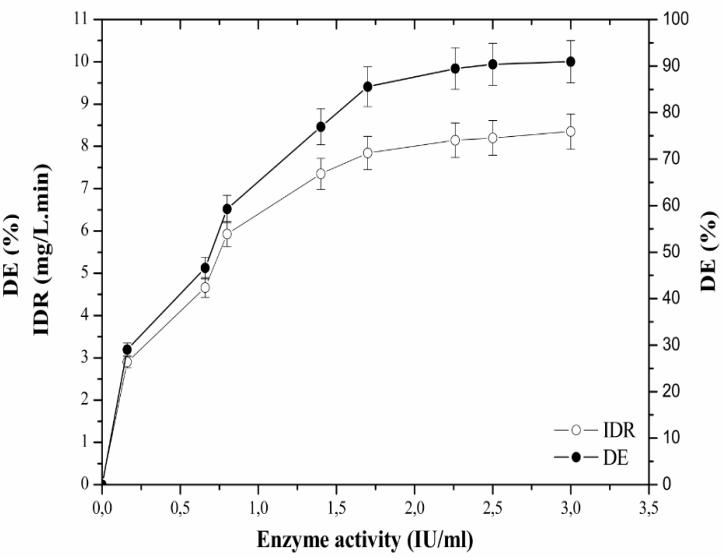

Fig. 5. Effect of enzyme activity on rate and efficiency of decolourization by C-peroxidase. (pH 2, T = 24 °C, 50 mg/L of DY106, 1 mM of H_2O_2).

into radicals which may further polymerize or degrade into small products (Kalsoom *et al.* 2015). So, from Fig. 4 is appeared that the DY106 decolourization efficiency and initial decolourization rate by C-peroxidase is affected by the concentration of hydrogen peroxide. It is clear that a concentration of 1 mM of H_2O_2 could be considered as critical dose. At lower concentrations the highest efficiency was noticed, while an inhibition effect took place at peroxide concentrations above 1 mM. The optimal of H_2O_2 dose will be taken as 1 mM. Comparable results were reported by using other plant peroxidases for decolourization of many azo dyes (Jamal *et al.* 2011). The optimal enzyme activity in the reaction medium was also determined. From Fig. 5 it is obvious that the efficiency and decolourization rate increased with increasing enzyme concentration to reach maximum values (89.5 %, 8 mg/L.min) when enzyme activity was above 2.25 IU/mL. No further improvement was noticed for higher enzyme concentrations. Mohan *et al.* (2005) obtained with horseradish peroxidase (HRP) activity of 2.2 IU/mL a maximum decolourization yield of 84 % of Acid Black 10 BX. After 2 min, the residual dye remained constant (DE = 89.45 %). The kinetics of enzymatic treatment of DY106 solution was then followed under optimal pH, dye

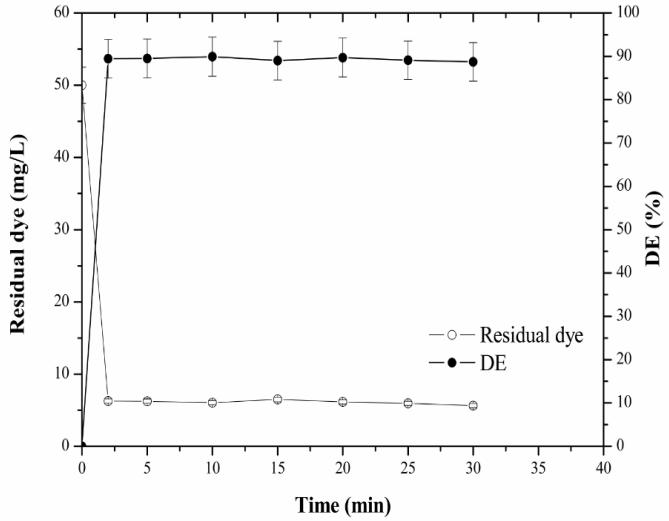

Fig. 6. Effect of contact time on residual dye and DE after enzymatic treatment by C-peroxidase. (T = 24 °C, 50 mg/L of DY106, 1 mM of H_2O_2 and 2.25 IU/mL of C-peroxidase).

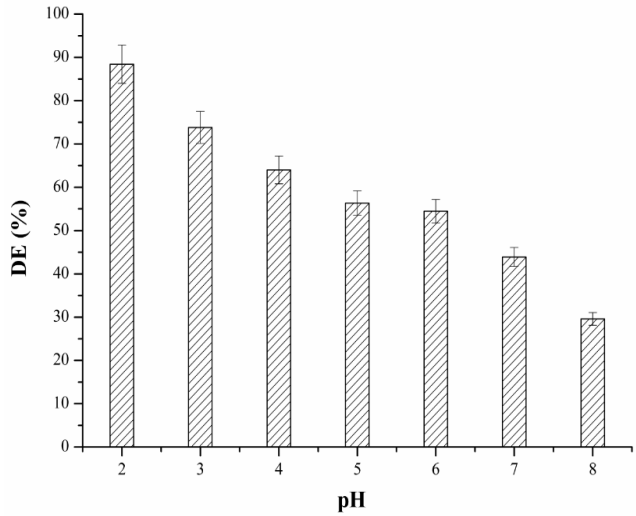

Fig. 7. Effect of pH on dye decolourization by Fenton treatment. (T = 24 °C, 50 mg/L of DY106, 1 mM of Fe^{2+}, 2 mM of H_2O_2).

concentration, H_2O_2 dose and enzyme activity. At regular time intervals, one vial was removed and analyzed for the residual dye concentration (Fig. 6). A contact time of 2 min for enzymatic treatment will be considered for the rest of the study

Fenton treatment, optimum pH, H_2O_2, Fe^{2+}, initial dye concentrations and time duration

It is well known that pH is an important parameter in Fenton process. It controls the production of hydroxyl free radicals and the concentration of ferrous ions (Sun *et al.* 2007) as illustrated in Eq. 1. From Fig. 7 it is clear that a degradation yield of 88.7 % was obtained at pH 2 at constant concentrations of H_2O_2 (2 mM) and Fe^{+2} (1 mM), respectively. The degradation of DY106 was significantly influenced by pH. Decolourization decreases with increasing pH. Similar results were obtained with different types of dyes and it was proved that maximum degradation was achieved at pH ranges between 2 – 4 (Neamtu *et al.* 2003; Meric *et al.* 2004; Sun *et al.* 2007). The other interesting aspect of this result is the concordance with optimal pH range of the enzymatic process, which facilitates the design of the combined process without the necessity of pH adjustment.

Data on Fig. 8 illustrate the relationship between decolourization efficiency (DE) and initial concentration of H_2O_2. The selection of an optimal H_2O_2 concentration for the degradation of the dyes by Fenton's reaction is important from an economical and practical point of view (Neamtu *et al.* 2003). Results indicate that the decolourization of DY106 increased with increasing H_2O_2 concentration from 0.5 to 8 mM. No significant increase was noticed for higher concentrations up to 16 mM. Sufficient amount of hydroxyl radicals were produced leading to higher decolourization yield (Panda *et al.* 2011). The optimal H_2O_2 dose giving maximum decolourization (93.7 %) was 8 mM.

Iron in its ferrous and ferric forms acts as a catalyst and requires a working pH below 4 (Nidheesh *et al.* 2013). In this work, concentrations of Fe^{2+} were varied to obtain the optimal concentration for Fenton treatment. Results are shown in Fig. 9.

The decolourization of DY106 solution increased with initial catalyst concentration till it reached 98.6 % when (Fe^{2+}) was 2.5 mM. Further increase of (Fe^{2+}) dose leads to the decrease of decolourization yield to reach 78.8 % at ferrous concentration of 10 mM.

Dye removal is directly proportional to catalyst (Fe^{2+}) concentration. This is mainly caused by the increase of $HO^{\bullet}$ radical concentration, which promotes the degradation efficiency of pollutants. Nevertheless, many studies have revealed that the use of excess concentration of Fe^{2+} could lead to the self-scavenging of $HO^{\bullet}$ radical by Fe^{2+} and thus induce the decrease in degradation rate of pollutants (Joseph *et al.* 2000).

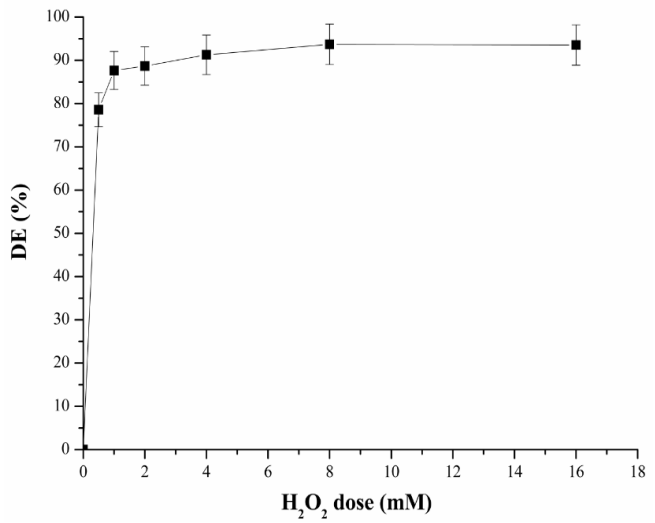

Fig. 8. Effect of H_2O_2 dose on dye decolourization by Fenton treatment. (pH 2, T = 24 °C, 50 mg/L of DY106, 1 mM of Fe^{2+}).

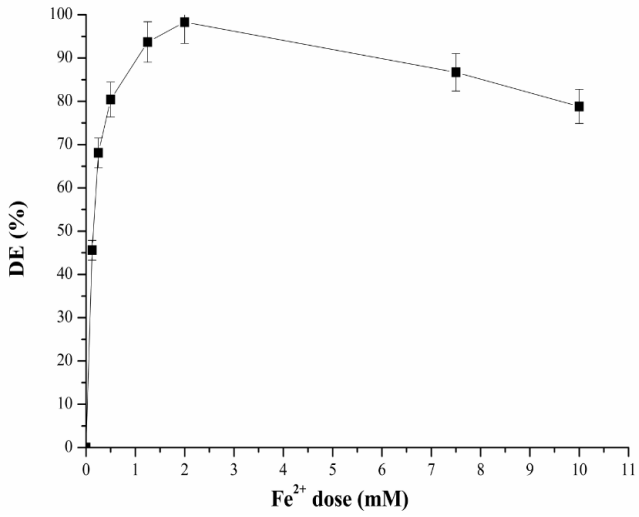

Fig. 9. Effect of Fe^{2+} dose on decolourization by Fenton treatment. (pH 2, T = 24 °C, 50 mg/L of DY106, 8 mM of H_2O_2).

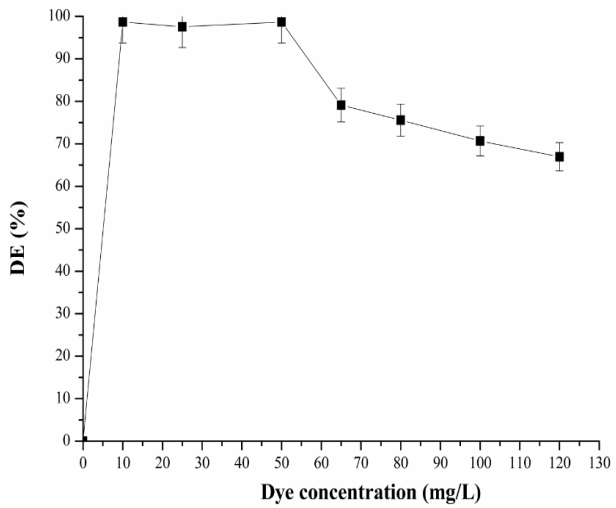

Fig. 10. Effect of initial dye concentration on decolourization by Fenton treatment. (pH 2, T = 24 °C, 2.5 mM of Fe^{2+}, 8 mM of H_2O_2).

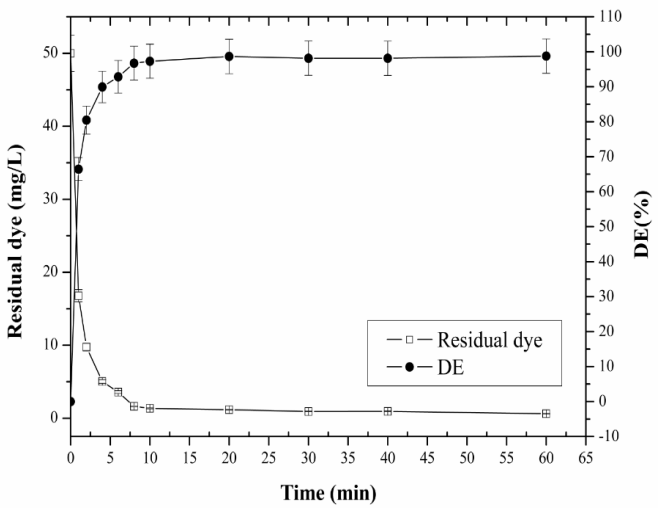

Fig. 11. Effect of time duration on DY 106 decolourization by Fenton treatment. (pH 2, T = 24 °C, 50 mg/L of DY106, 2.5 mM of Fe^{2+}, 8 mM of H_2O_2).

Initial concentration of dye is an important parameter in practical applications (Modirshahla *et al.* 2007). Therefore, the effect of dye concentration on the decolourization efficiency was investigated at different initial concentrations of DY106. Results are presented in Fig. 10.

We observed that the decolourization efficiency decreased slowly with increasing the initial dye concentration. The highest efficiency (98.7 %) was obtained for DY106 concentrations below 50 mg/L. For higher concentrations, the yield decreased. This could be explained by the unbalanced concentrations of dye and hydroxyl radicals for a fixed iron concentration. Therefore, the optimal dye concentration was taken as 50 mg/L. Fig. 11 provides an effect of reaction time duration on DY 106 decolourization and dye removal efficiencies by Fenton reaction.

Further, it was noticed that decolourization increases with contact time under optimal conditions. The residual DY106 concentration decreased rapidly during the first 10 min (DE =98.6 %) and then slowed down as time goes on. Similar results were obtained by Sun *et al.* (2007) for decolourization of 50 mg/L of Amido black 10B (DE= 69.3 %) under specified conditions.

Combined Enzymatic- Fenton treatment

The major purpose of this integrated process was to reduce the operational concentration of reagents, particularly the ferrous ions concentration used in Fenton's reagent, to achieve the highest decolourization efficiency of DY106.

Data indicate that both enzymatic and Fenton processes as individual treatments occur under similar conditions of pH (2), ambient temperature, optimal dye concentration (50 mg/L) in the presence of hydrogen peroxide in each enzymatic and Fenton treatment. Similarities in mechanisms of both processes could be also exploited for the development of a hybrid process in order to reduce the dose of iron in Fenton process (less than 2.5 mM), alternatively, improve decolourization efficiency of enzymatic process (the efficiency was 89.5 %).

The reduction of certain by-products e generated by either Fenton or enzymatic processes could be also performed by sequential or mixed processes. For this purpose, the effect of Fe^{2+} for all combined processes was studied under constant conditions of H_2O_2 dose (8 mM), enzyme activity (1.7 IU/mL), pH 2, temperature (24 °C) and dye concentration (50 mg/L). On the other hand, contact time was optimized for mixed combined process.

As seen in Fig. 12, the decolourization capacity was significantly affected when Fe^{2+} concentrations were below 1 mM, for all combined process under specific conditions. It was also noticed that the sequential Fenton-enzyme treatment (FE) and mixed process, were more efficient when Fe^{2+} dose varied from 0.125 to 1.25 mM, which gives

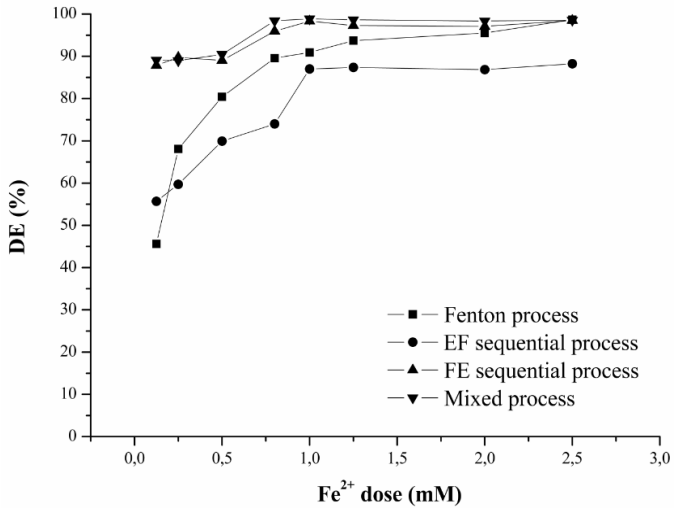

Fig. 12. Effect of Fe^{2+} dose on decolourization efficiency by Fenton and different combined processes. (pH 2, T = 24 °C, 50 mg/L of DY106, 8 mM of H_2O_2 and 1.7 IU/mL of C-peroxidase).

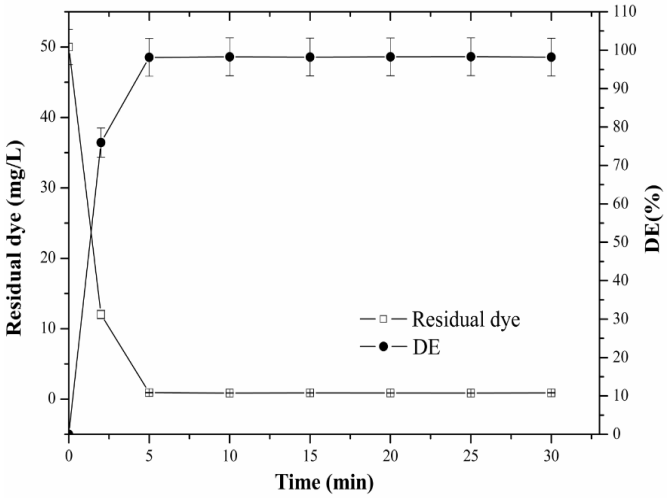

Fig. 13. Effect of time duration on decolourization efficiency by mixed process. (pH 2, T = 24 °C, 50 mg/L of DY106, 0.8 mM of Fe^{2+}, 2 mM of H_2O_2 and 1.7 IU/mL of C-peroxidase).

efficiency between 88 and 98 %. For Fe^{2+}concentrations above 1 mM, efficiencies of FE, mixed and Fenton processes were comparable. A similar result (91 %) was obtained for sequential biological Fenton process for decolourization of Remazol red with Fe^{2+} dose of 1 mM (Jonstrup *et al.* 2011). Finally, the effect of contact time on decolourization efficiency for mixed process (Fig. 13) showed that more than 98 % of colour was eliminated in 5min. According to kinetic data shown on Fig. 6 and Fig. 11, enzymatic process was faster than Fenton treatment. This indicates that during mixed treatment, the enzymatic process starts firstly and may generate specified, readily degradable products by Fenton reaction. No comparison could be shown with results that were obtained by sequential chemical-biological process which needs 39 min for Fenton and 72 days for the enzymatic treatments (Mandal *et al.* 2010). Several reviews (Guieysse and Norvill 2014) confirmed this observation. Recapitulation of optimal treatment conditions by all processes and efficiency (DE) are listed in the Table 1.

Table 1. Optimal conditions and efficiencies of different treatment processes for the DY106.

Process	Enzyme	Fenton	F-E	E-F	Mixed
pH	2	2	2	2	2
Dye [mg/L]	50	50	50	50	50
H_2O_2 [mM]	1	8	8	8	8
AE [IU/mL]	2.25	00	1.7	1.7	1.7
Fe^{2+} [mM]	----	2.5	1	1	0.8
Contact time [min]	2	10	20	20	5
DE [%]	89.5	98.6	98.3	87.0	98.4

UV-visible spectra and HPLC analysis of the DY106 treated by different chemical enzymatic process

Despite the technical performance results that were obtained in terms of DE, the treated solutions were also analyzed by UV-visible spectroscopy and HPLC chromatography in order to compare the qualitative efficiency of each combined treatment. Analysis was performed on samples before and after treatment under optimal conditions: pH 2, 8 mM of H_2O_2, 0.8 mM of Fe^{2+} and 50 mg/L of DY106.

Before treatment of DY106 aqueous solution (50 mg/L), UV-visible spectra consisted of two main characteristic absorption bands (Fig. 14). One is in UV region at 212 nm and another in visible region at 396 nm. UV band is characteristic of adjacent rings (transition $\pi \rightarrow \pi^*$), whereas visible band owns to long conjugated π system linked by azo groups (transition $n \rightarrow \pi^*$) (Silverstein *et al.* 1991). It is clear that the adsorption peak at 396 nm completely disappeared after each process treatment. This indicated that under optimized experimental conditions (pH 2, Fe^{2+} dose of 0.8 mM, H_2O_2 dose of 8 mM and DY106 concentration of 50 mg/L)

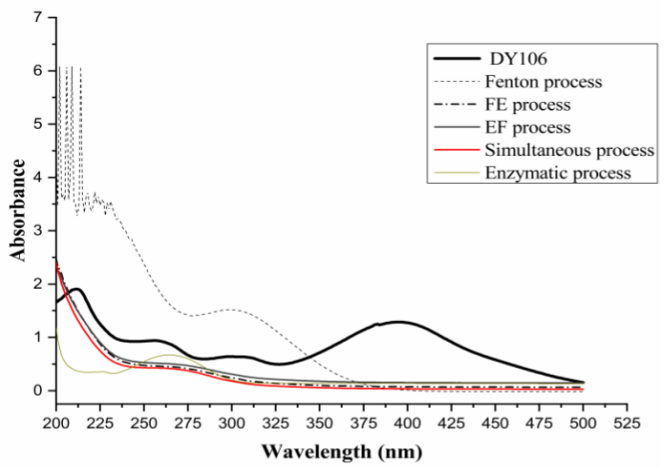

Fig. 14. UV-visible spectra of DY106 untreated and treated solutions by: C-peroxidase, Fenton, sequential and mixed combined process. (pH 2, T = 24 °C, 50 mg/L of DY106, 0.8 mM of Fe^{2+}, 8 mM of H_2O_2 and 1.7 IU/mL of C-peroxidase).

the chromophore azo group -N=N- was destructed. In the UV zone, the disappearance of the band at 212 nm was also observed, but other cases could be discussed such as the appearance of a new peak at 265 nm after enzymatic treatment probably due to the formation aromatic amine intermediate or other products (Hailei *et al.* 2009), strong absorption bands at 300 nm and between 200 and 250 nm after Fenton treatment or complete disappearance of DY106 characteristic bands after sequential FE, EF and mixed combined processes, with minimum absorption for the mixed combined treatment (compared to the sequential one).

Further, with Fenton oxidation, results showed that the destruction of the aromatic rings was difficult. This could be due to the lowest energy band which is assigned to the n→π* transition related to the azo group. Therefore, HO• radical attacks firstly the –N=N– group of the lowest energy leading to the opening of the –N=N– double bonds. The long conjugated π systems were then destructed, and consequently a maximum of decolourization was achieved (El-Desoky *et al.* 2010). Also, the absence of bands in the UV zone after treatment by combined processes shows that the by-products which were generated after the first treatment were eliminated during the second one.

The oxidation of aromatic amines by bitter gourd peroxidase and Fenton's reagent was previously thoroughly studied (Karim and Husain 2009; Casero *et al.* 1997). This is clearly confirmed in Table 2 which compares retention time and peak areas of solutions before and after treatment by combined processes. Results show that there is a certain synergy between the two processes which conducted to the elimination of compounds which were generated by individual processes after sequential or mixed processes. The synergetic aspect could be also demonstrated by further analysis with NMR spectroscopy and mass spectrometry.

Phytotoxicity studies

Coloured textile effluents pose serious health and environmental problems. They can be rejected directly and thus return to agricultural or industrial activities. Thus, the importance of conducting phytotoxicity tests for enzyme, Fenton and combined treated processes and untreated dye solutions, in order to evaluate the possible use of the treated aqueous solutions in the irrigation or industrial fields, is appeared.

Results in Table 3 show that the germination of *P. vulgaris* L. in the case of the treated DY106 (100 ppm) by simultaneous peroxidase-Fenton, did not significantly differ from their germination in distilled water (96.66 % vs. 95.03 %). At the same found to be lower in the case of germinated seeds by Fenton, enzyme and untreated solutions than seeds germinated that were irrigated with distilled water and simultaneous peroxidase-Fenton treated solution. Moreover, germination was also influenced by the quality of the effluent that was

Table 2. Retention time and peak areas of HPLC spectra of DY106 and treated solutions.

Process	Retention time [min]	Peak area [mV.s]
DY 106	2.242	1444000
	4.025	2770254
	6.817	69299
Enzyme (E)	2.433	72301
	3.075	884384
Fenton (F)	2.997	985432
	5.013	83532
F-E	2.292	427785
	3.050	688916
E-F	2.283	503835
	3.033	347095
Mixed	2.283	474616
	3.042	438942

Table 3. Phytotoxicity tests on growth of P. *vulgaris* L. irrigated in the absence or presence of raw or treated DY106 solutions.

Growth parameters	Distilled water	DY106 [100 ppm]	Enzymatic treatment	Fenton treatment	Mixed treatment
Germination [%]	96.66	28.46	46.92	63.85	95.03
Radicle [cm]	5.30±0.3	1.09±0.1	2.28±0.2	1.57±0.1	3.89±0.4
Plumule [cm]	11.75±0.2	6.66±0.3	6.21±0.3	7±0.5	10.42±0.2

generated after individual treatments (63.85 % for Fenton *vs.* 46.92 % for peroxidase). This indicates that the azo-dye DY106 was more toxic than degraded products obtained after Fenton or enzymatic treatments. Alternatively, treated water from combined processes could be considered as non-toxic, which could help promoting it´s reuse.

Conclusions

From the findings of this work it can be concluded that C-peroxidase and Fenton reagent have potential in the decolourization of DY106 under specified experimental conditions. The decolourization performance by enzymatic treatment depends on pH, enzyme activity and initial dye concentration. Similarly, Fenton process efficiency was affected by pH, H_2O_2, Fe^{2+} and dye concentrations, H_2O_2 and Fe^{2+}. Optimal operating conditions regarding yield and decolourization rate were determined for both enzymatic and Fenton processes.

Applied individual processes present some drawbacks; these include high ferrous dose in the case of Fenton process or the generation of toxic or unknown compounds even in the case of enzymatic treatment. Due to similarities existing between enzymatic and Fenton processes (pH 2 and the presence of hydrogen peroxide and iron as key elements, and optimal dye concentration), the mixed and sequential combined enzymatic-Fenton processes resulted in improvements such as the reduction of ferrous dose, minimizing degradation time and increasing yield. Elimination of (possible) by-products of the Fenton process showed that there is a certain synergy with enzymatic process. Thus, combined approaches were effective in degrading components of the colored solutions, especially at lower iron concentrations, with comparison to sequential processes.

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