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HPLC-MS/MS Mechanistic Study of Direct Yellow 12 dye Degradation Using Ultraviolet Assisted Ozone Process

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ABSTRACT

HPLC-MS/MS degradation mechanism of Direct Yellow 12 (DY-12) dye using O₃ associated with UV was studied. The influent of different conditions such as pH, initial DY-12 dye concentration and reaction time were studied in a batch reactor method. The results revealed that the pH value and DY-12 initial concentration controlled the removal process. The maximum color removal was achieved in alkaline condition (pH 9) as compared to neutral or acidic conditions. The color removal of DY-12 dye followed the first-order kinetics. When UV was applied with ozone simultaneously, the first order rate constant (*kd*) increased, and the time of dye decolorization shortened to 10 min for 200 ppm dye concentration. These results indicated that the application of UV can reduce the reaction time and dose of ozone. Gas chromatography-mass spectrum and HPLC-MS/MS analyses of the treated synthetic dye solution at the end of the treatment time showed no toxic organic compounds were detected. The COD decreased by more than 85% of the initial COD of the untreated DY-12 dye concentration.

Keywords: Advanced Oxidation; Decolorization; Detoxification; Direct Yellow 12 Dye; HPLC-MS/MS; Ozonation

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INTRODUCTION

Colored effluents from industries present environmental problems via absorbing light and interfere with the aquatic biological processes [1,2]. Therefore, it is very important to reduce the discharge of colored effluents into the ecosystem and enforcement of the effluent color standard should become a concern for legislators in all countries. The industry of textile has traditionally been one of the most important industries in Egypt and is considered one of the major water consumer industries, which releases a huge quantity of wastewater effluents during handling, particularly, in the washing and coloring steps. The wastewater encompasses great amounts of both organic matter

and colorants (dyes) [1]. Environmentally, these pollutants mean suspended solids, COD, BOD, as well as high pH and strong color. The elimination of dye color in the treatment of dye manufacture and textile dyeing wastewaters is one of the core problems of textile industry [2].

In addition, there are many various types of dyes and several dyeing manufacture procedures, making difficult that only a single treatment process answers satisfactorily to the treatment requirements in all circumstances [3]. Direct dye is still the most widely used in dyeing printing process of the textile industry. Large volumes of synthetic dyes are consumed by various industries such as textile dyeing (60%),

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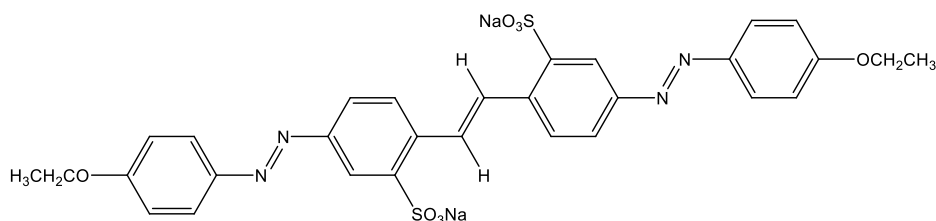
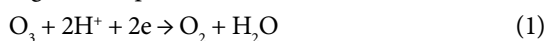


Fig. 1. Chemical structure of Direct Yellow 12, Chrysophenine G (Molecular Formula: $C_{30}H_{26}N_4Na_2O_8S_2$) (Molecular Weight: 680.66). Soluble in water solution for yellow until golden brown, adequate soluble in ethanol and slightly soluble in acetone for green light yellow. $\lambda_{max} = 518$ nm; C.I. 24895.

plastic matter (10%), and paper (10%) [4].

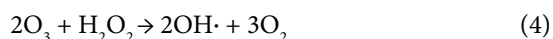
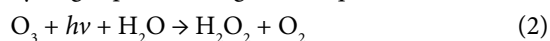
The presence of minor amounts of dyes in water (sometimes <1 ppm for some dyes) is highly noticeable and unacceptable [5]. Synthetic dyes are poisonous and carcinogenic and also common water contaminants causes a severe threat to aquatic living organisms [6]. Though, wastewater comprising dyes is hard to treat, since the dyes are recalcitrant organic molecules, resistant to aerobic digestion, and are stable to light, heat and some oxidizing agents [7].

The conventional approaches for wastewater treatment, for instance, reverse osmosis, biotreatment, carbon adsorption, and ozone oxidation treatment experience several limits [8]. It shows that neither these biological treatments nor normal chemical methods can outcome in acceptable amounts of decolorization and COD reduction [9]. Different activated carbons have been used for the color removal of direct dyes [10-13]. The adsorption and photocatalytic removal of different dyes have been reported [14-16]. Advanced oxidation processes (AOPs) assumed was encouraging methods for the treatment of harmful, noxious organic contaminants in aqueous solutions [17-20]. The production and use of powerfully but fairly non-selective hydroxyl radicals in adequate quantities to be able to oxidize most of the organic compounds existing in the industrial wastewater [8]. Ozone is a powerful oxidizing agent (E^0 1.78 V) and can be used instead of hydrogen peroxide (E^0 2.07 V) in the textile effluent treatment as a very attractive alternative with considerable application potential. Eq. (1) is considered a strong oxidant of inorganic and organic compounds [21].



Ozone can be reacted with several classes of organic compounds through direct or indirect reactions causing accepted degradation. Dyes Chromophore is known as organic compounds with conjugated double bonds, which can be broken directly or indirectly by ozone into smaller forms of organic compounds causing the color removal of the dyes effluent [22]. Photolysis of ozone in water in the presence of UV

radiation (200-280 nm) led to the formation of hydrogen peroxide as given in equations 2 to 4:



Low-pressure mercury vapor UV lamps are commonly used as a source of UV irradiation used in this process. Different parameters such as the presence of scavengers in the influent, the pH value, the wastewater turbidity, temperature of the process, intensity of the UV lamp and the pollutant type(s) are affecting the efficiency of the treatment system [23-26]. The major goal of this work was to investigate the application of O_3 and UV/ O_3 processes for degradation and detoxification of Direct Yellow 12 dye in a laboratory-scale reactor system for decolorization. HPLC-MS/MS study of the mechanism of dye degradation. The removal of COD and the toxicity of the treated wastewater were also investigated.

MATERIALS AND METHODS

Direct Yellow 12 was purchased from ISMA dye company Kafer Eldawar, Egypt ~75% of dye content and was used without further purification. Chemical structure of DY 12 dye is shown in Fig. 1. A stock of 1000 mg/l of DY-12 were prepared using double distilled water and other working aqueous solutions used (100, 200, 300, 400 and 500 mg/l) of DY-12 were obtained from the stock solution by dilution in double distilled water to the required concentration. DY 12 dye concentrations in the solution were obtained by comparison with standard solutions in the visible range at the λ_{max} 518 nm [27] using UV-VIS spectrophotometer (Analyticjena Spekol 1300 UV VIS Spectrophotometer, Cole Parmer Instrument Co., USA).

The color removal rate of DY-12 was determined by Eq. 5.

$$Color\ removal\ efficiency\ \% = \frac{A_0 - A_t}{A_0} \times 100 \quad (5)$$

Where A_0 and A_t are the initial and the measured absorbance of the samples at different time intervals.

Treatment was done in a 500 ml glass reactor and all links between the Ozonator and the reaction vessels were made of a tube of Teflon. The Ozonator Model: N 1668A power: 18W Vol AC 220V/50HZ was used to generate ozone with a concentration of 500 mg O_3 per hour. The ozone that leaves the reactor was caught into tow-sequential bubblers filling with 2% KI solution. Also, experimental process of O_3 /UV was performed in Laminar Floe Cabinet (Bw-LFH1300) containing 254 nm UV lamp with 30W power.

For the chemical oxygen demand determination according to the procedure stated in APHA 2005 [28]. The COD was calculated for both untreated and treated DY-12 at concentration 500 mg/l.

Influence of the pH was studied by adjusting the reaction mixture to different initial pH (3 to 11) value using 1M H_2SO_4 or 1M NaOH before starting treatment processes. The pH meter (JENCO Electronics, LTD, Model: 6173, Serial No: JC 05345) was used for the pH adjustment.

GC instrument (Agilent Technologies 7890A) equipped with an HP5 MS column (30 m \times 250 μ m \times 0.25 μ m film thickness) and mass detector (Agilent Technologies 5975C) was used to study the final degradation products using benzene as the extraction solvent [29]. The temperature of the injector was 290°C (splitless). Other Chromatographic conditions included an initial oven temperature of 90°C, with a 61.2 min isotherm and a program rate of 8°C min^{-1} and a final oven temperature of 300 °C for 30 min. The gas carrier was He, with a column flow of 1.5 ml min^{-1} .

LC-MS/MS Agilent Technologies 1260 infinity connected with Agilent Technologies 6420A Triple Quad Mass detector and Eclipse Plus C18 column (4.6 \times 100 mm, 3.5 μ m) was used for degradation mechanism study. The solvent used as mobile phase was acetonitrile: water with 1% formic acid 50:50 (vol/vol). The flow rate was 0.5 mL min^{-1} , and 5 μ L of the sample solution was injected. The flow rate was 0.5 mL min^{-1} , and 5 μ L of the sample solution was injected. The mass spectrometer was equipped with an electrospray ionization (ESI) source and operated at negative polarity. The ESI conditions were as follows: capillary voltage, 4.0 kV; the nebulizer pressure, 35 psi; drying gas flow, 10 L min^{-1} ; temperature, 325 °C. The mass range was from 90 to 700 m/z .

Toxicity Assay

For evaluating the toxicity of the DY-12 dye and its degradation products on the zooplankton, Rotifer *Bruchionus plicatilis* toxicity experiments were implemented on both untreated and the 180 min UV enhanced ozone treated solutions following the recommendations of Standard Methods [30,31]. The animal test was obtained by hatching rotifer cysts to produce animals of similar age, genotype and similar physiological condition to greatly reduce test variability. The controlled cyst hatching was achieved by transferring to lower salinity, warmer temperature, and light. After 23 h at 25°C and 15‰ salinity, hatching begins and proceeds rapidly. By 28 h, about 90% of the cysts have been hatched [32]. Toxicity assay was done by using neonates of Rotifer *Bruchionus plicatilis*. 40 ml of DY-12 dye with concentration (500 mg/l) completed to 200 ml seawater each ml of sea water contain 20 neonates of Rotifer *Bruchionus plicatilis* for treated and untreated samples. The same volume of normal seawater was used in control wells. The number of live and dead neonates in every well was calculated using a microscope (Nikon Eclipse E200) at different time intervals of 0, 15, 30, 45 and 60 minutes.

RESULTS AND DISCUSSION

Effect of pH value

Under alkaline conditions ozone mainly decompose to hydroxyl radicals ($\cdot OH$) which can react easily with the target organic contaminant. On the other hand, under acidic conditions ozone can mainly react directly with organic contaminants [33]. Fig. 2 illustrates the color removal of DY-12 dye under different pH conditions. Fig. 3 shows that the color of DY-12 dye reduced from the initial concentration of 100 ppm to below 30 ppm within first 30 minutes only when ozone was provided at

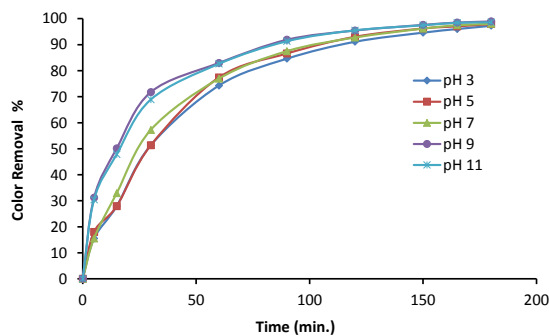


Fig. 2. Color reduction of the Direct Yellow 12 versus contact time at different pH (3, 5, 7, 9 and 11), (Dye concentration = 100 mg/l, O_3 = 500 mg/h, treated volume = 200 ml).

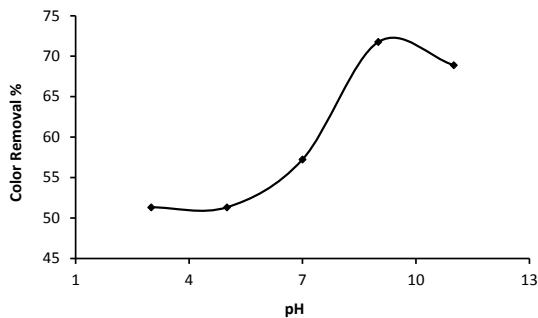


Fig. 3. Effect of Different pH (3, 5, 7, 9 and 11) on decolorization of Direct Yellow 12 at first 30 minutes, (Dye concentration = 100 mg/l, O_3 = 500 mg/h, treated volume = 200 ml).

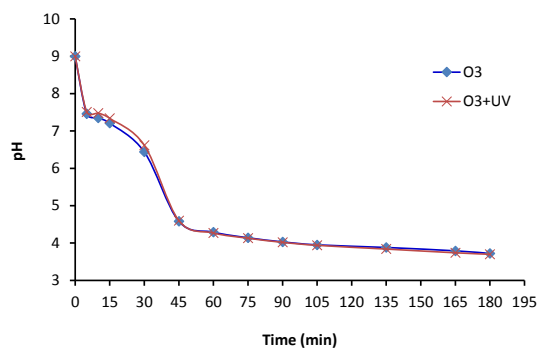


Fig. 4. pH monitoring during ozonation and advanced oxidation processes (O_3 = 500 mg/h, treated volume = 200 ml, initial solution pH 9 and 100 mg/l dye concentration).

500 mg/h at pH 9. Fig. 4 shows the change in the initial pH of DY-12 dye concentration of 100 ppm from 9 to 3.72 at the complete color removal. The highest decolorization rate for DY-12 dye occurred under alkaline condition (pH = 9). Ozone controlled the degradation process at lower pH value, whereas the hydroxyl radical showed a more considerable effect at higher pH value [34]. As a result of the sluggish decay rate (i.e., O_3 react with less plentiful OH^-) at lower pH, O_3 would build up in the liquid phase to an elevated concentration (i.e., higher O_3 saturation concentration at acidic pH). Moreover, as a result of the influence that extra massive reactions of O_3 with OH^- happen at higher pH value, stable state concentration of O_3 in the water is decreased while more hydroxyl radicals ($\cdot OH$) are generated at higher pH value [34]. The above-mentioned relationships influence the color removal in liquid phase following the coming two ways: (1) O_3 molecule controls the oxidation process of the organic contaminant at lower pH value, and (2) hydroxyl radical ($\cdot OH$) controls the oxidation process of the organic contaminant at higher pH value.

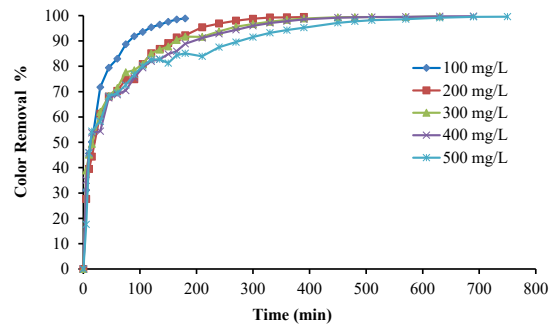


Fig. 5. Effect of ozone one different Direct Yellow 12 concentrations (100, 200, 300, 400 and 500 mg/l) versus time. (O_3 = 500 mg/h, treated volume = 200ml, initial solution pH 9).

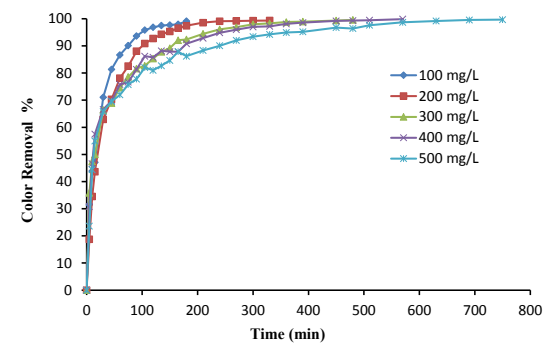


Fig. 6. Effect UV assisted ozone on different Direct Yellow 12 concentrations (100, 200, 300, 400 and 500 mg/l) versus time. Ozonation (O_3 = 500 mg/h, treated volume = 200ml, initial solution pH 9).

Hydroxyl radicals ($\cdot OH$) have a higher oxidizing potential and also, are less selective than molecular ozone O_3 for attacking the DY-12 dye, leading to a decrease in the rate of decolorization and the rate of mineralization increase at high pH values [35-37]. Thus, the solution pH plays an important role in ozonation process. It should be noted that the structure of the dye also plays an important role and can affect the rate of decolorization by oxidation of hydroxyl radical or ozone. As in the instance of DY-12 dye where the maximum color removal happens at pH 9. This research work indicated that a suitable pH regulation might be mandatory at the end of the treatment process.

It is also worth mentioning that the pH of the solution decreases rapidly at the early stage of the experiment, dropping from the initial solution (pH 9 at $t = 0$ min) to about 4.27 after 60 minutes ozonation (Fig. 4). Later, the decrease of pH continued more slowly until a steady state was reached at the end of the process. The drop of the pH indicated the formation of acidic by-products, such as H_2SO_4

originating from the sulfonate groups and a lesser extent, from the formation of aliphatic acids. It has to be noted that the formation of aliphatic acid compounds is usually encountered while dealing with ozonation of benzene derivatives [38].

Effect of initial dye concentration

It is an important from the practical point of view, to investigate how the initial dye concentration can influence the color removal. Thus, the influence of DY-12 dye (100–500 mg/l initial concentrations) on color reduction was examined. The effect of DY-12 dye initial concentration on decolorization efficiency is shown in Figs. 5 and 6. The decolorization of DY-12 dye by UV enhanced ozonation are slightly more effective than ozonation alone as mentioned by Peternel et al. [39]. Fig. 5 shows the color reduction effectiveness of DY-12 in different initial concentration. The color reduction effectiveness for the concentration values of 100, 200, 300, 400 and 500 mg/l in first 180 min were 98, 92, 91, 88 and 85%, respectively for O₃ only. This could be that in (AOPs), by increasing the dye concentration, many numerous intermediates arisen after the parent dye degradation which can interfere with the oxidation process. Such suppression would be more noticeable in the existence of an elevated amount of degradation intermediates that arisen after increasing in dye concentration [26]. Therefore, the required time to complete color removal should be increased with increasing of the initial dye concentrations. Fig. 6 shows the color reduction effectiveness for concentration values of 100, 200, 300, 400 and 500 mg/l in first 180 min were 99, 97, 92, 88 and 87 respectively for UV assisted O₃.

The degradation efficiency decreased as the initial dye concentration increased which may be attributed to the fact that the ozone dose is stable. It is seen from Figs 5 and 6 that when to increase the initial dye concentration the color removal rate is lowered and need longer time to achieve the complete color removal. This because of the ratio of dye molecules to ozone molecules in the solution under the practical conditions decreased with increasing of the initial dye concentration [35]. A similar result was reported by Sevimli and Kinaci [40].

Promotion Effects of UV on DY-12 dye degradation

From Figs. 5 and 6 it was shown that the time needed for complete decolorization of DY-12 dye in the case of UV + O₃ processes was slightly less

than the time for O₃ alone. But it is clear to notice that in 200 ppm concentration the time is decreased by 30 minutes to reach the same color removal 99.25%. This shows the better performance for dye degradation by UV + O₃ process in comparison to ozone alone. UV Enhanced O₃ in comparison to ozone alone can produce more hydroxyl radicals which more efficient for mineralization. The ozone is available as molecular O₃ in acidic pH, while it decomposes into secondary oxidants which one among them is hydroxyl radical that considered the most important one in alkaline pH. Yasar et al. [41] mentioned that the oxidation potential of ozone decreases in alkaline solutions which also, indicate the higher decomposition and lower solubility of ozone.

Chemical oxygen demand (COD) removal

COD test is commonly used to indirectly measure the number of organic compounds in the wastewater. After complete color removal (750 minutes of AOPs (UV+O₃)) more than 85% of the initial COD was eliminated for 500 ppm, which likewise with what was reported by Zhao et al. [42], the degradation of dyes with ozone commonly produces a little fragments of organic molecular such as ketones, epoxides, aldehydes, etc., that may lead to a remaining COD. Such COD developing from those few new produced compounds that can be later reduced by biological treatment methods [43].

$$COD \text{ removal rate } \% = \frac{COD_i - COD_t}{COD_i} \times 100 \quad (6)$$

$$COD_i = 968.4 \text{ mg/l and } COD_t = 142.8 \text{ mg/l}$$

Kinetic of Direct Yellow 12

The kinetic study of ozonation system plays an essential role in evaluating the effectiveness of treating process. The direct reaction of ozone with DY-12 dye is supposed to be first-order kinetic with regarding dye and ozone. However, in the presence of excess amount of ozone or when the ozone concentration is expected to reach a stationary phase at the interface, the rate of oxidation follows the first-order kinetics with regard to dye concentrations. During this work, the ozonation kinetics of DY-12 was estimated by drawing Ln(C_t/C₀) readings against the time of reaction using the equation 7:

$$\ln\left(\frac{C_t}{C_0}\right) = k_d t \quad (7)$$

Table 1. First order constant (k_d), $T_{1/2}$ and initial concentration of DY-12 dye in AOP comparison to ozonation alone processes (pH 11, UV lamp 254 nm, power = 30W, $O_3 = 500$ mg/h).

Initial concentration of DY-12 (mg/l)	k_d (min^{-1})		k_d (min^{-1})	
	$U_V + O_3$	$T_{1/2}$ (S)	O_3	$T_{1/2}$ (S)
100	0.0272	1529	0.0259	1605
200	0.0184	2260	0.0144	2888
300	0.0125	3327	0.0107	38886
400	0.0114	3648	0.0101	4117
500	0.0081	5134	0.0080	5198

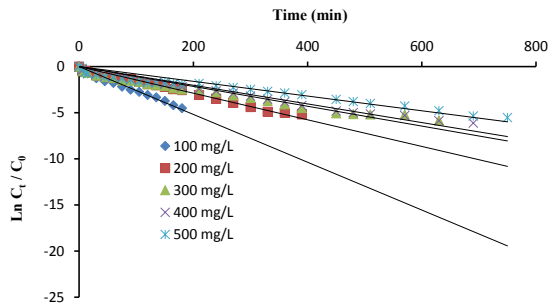


Fig. 7. Kinetics of the Direct Yellow 12 ozonation ($O_3 = 500$ mg/h, treated volume = 200 ml, initial solution pH 9).

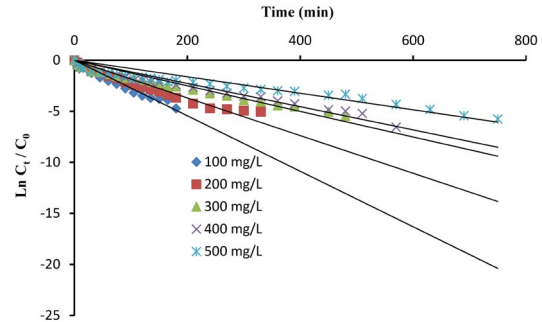


Fig. 8. Kinetics of the Direct Yellow 12 UV assisted ozonation ($O_3 = 500$ mg/h, treated volume = 200 ml, initial solution pH 9).

where C_t is the concentration of DY-12 dye through any reaction times (t) during the ozonation and C_0 is the initial concentration of DY-12 dye. k_d is the constant of the first-order reaction rate.

Figs. 7 and 8 report the plot of dye concentration removal versus contact time which was relatively quick in the first 5 minutes of both O_3 and O_3/UV , then; the dye fading speed was slower. In this work, it was noticed that the first order rate constant (k_d) decreased when the initial DY-12 dye concentration increased, in both the AOPs and ozonation process (Figs. 7 and 8). We indicate the first order performances were also seen through another ozonation process of azo-dyes [44]. The DY-12 decolorization by O_3 alone and UV assisted O_3 followed the first-order kinetics, agreeing with other reported studies [38, 45]. A series of k_d shown in Table 1, (from 0.0272 to 0.0081 min^{-1}) remained slightly higher than those (from 0.0259 to 0.0080 min^{-1}) attained in ozonation processes. According to the dynamics theory, $T_{1/2}$ represents the time of DY-12 dye decolorized to its half of initial concentration. For the first order reaction, the readings of $T_{1/2}$ could be calculated from equation 8:

$$T_{1/2} = \text{Ln}\left(\frac{2}{K}\right) \times 60(\text{s}) \quad (8)$$

Compared to the ozonation process alone, $T_{1/2}$

shortened 10.5 min at an initial concentration of 200 mg/l, and the whole reaction time decreased by 22% (Table 1), as mentioned before that the presence or absence of UV radiation hardly affects the decolorization rate [46].

Identification of the Intermediates byproducts of DY 12 during the AOP

It is desirable that the ozonation decomposed final products be safe, but the intermediates and end products after ozonation of dyes would have poisonous influences [47-49]. Therefore, identification and/or ecotoxicological assessment of such intermediates and final treatment products have to be done. As reported before [50-52], a lot of earlier data indicate that the intermediates and end products of ozonation rely on the dye parent compounds chemical structure, in addition to the treatment circumstances.

The GC-MS study of ozonated textile wastewater (pH 9) after 180 and 210 minutes showed that it is obvious that AOPs lowered considerably the substances of the organic textile wastewater extracts. A preliminary analysis of the ozonated synthetic wastewater samples showed no aromatic amines or other toxic substances are reported by GC-MS chromatogram. Somensi et al. [53] stated that certain aromatic compounds can react quite slowly during ozonation process and will need many times of treatment to degrade. During the experimental

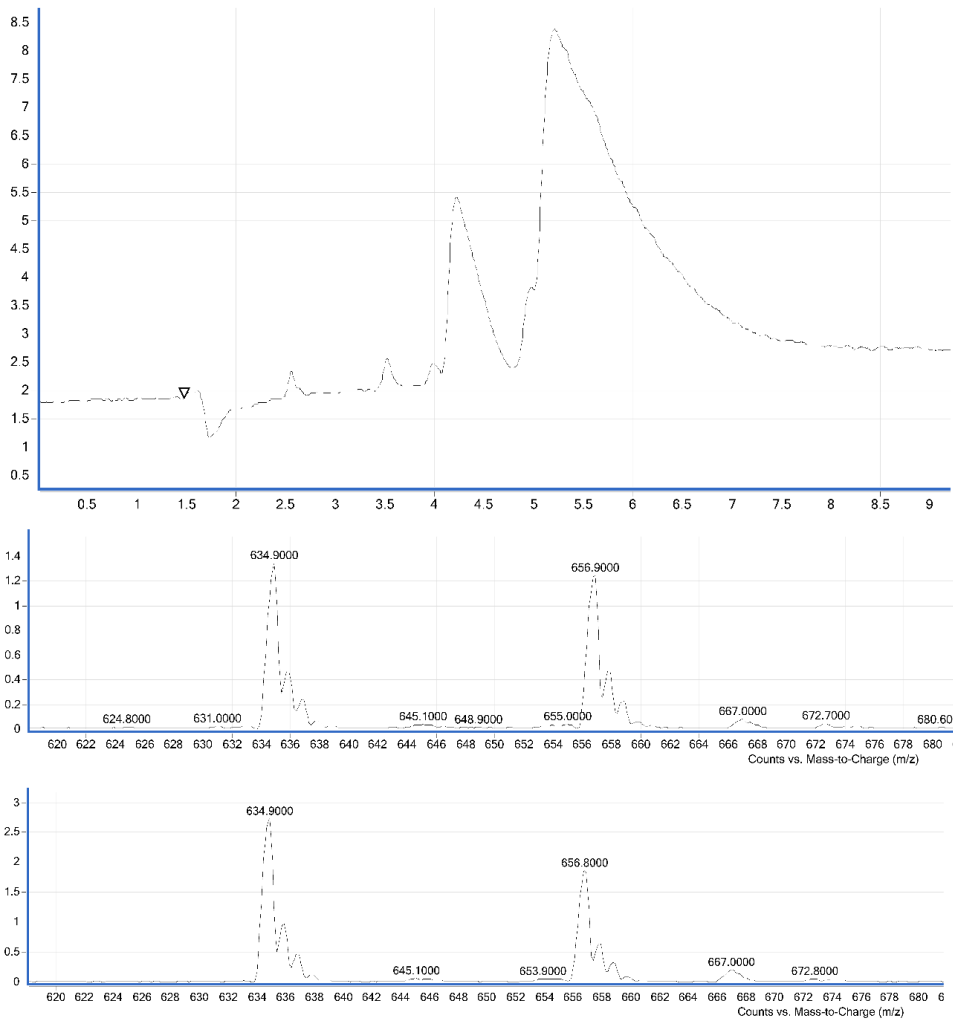


Fig. 9. HPLC-MS/MS analysis of DY-12 dye solution using MS2 Scan mode.

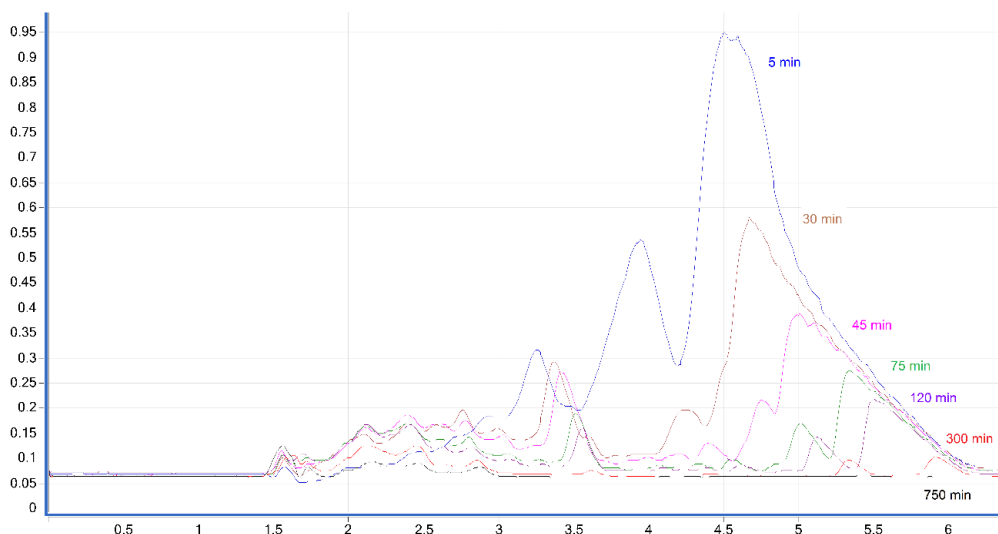


Fig. 10. HPLC-MS/MS analysis of DY-12 dye degradation using AOP at different time interval (5, 30, 45, 75, 120, 300 and 750 minutes).

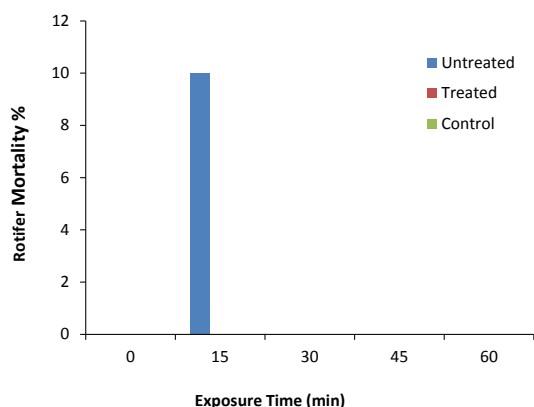


Fig. 11. Toxicity of the raw and ozonated solutions at different exposure time (Dye concentration = 100 mg/l, O_3 = 500 mg/h, treated volume = 200 ml, ozonation time = 180 min, pH = 7.50).

circumstances operated in this work we extend the time for ozonation for 30 minutes more and we notice that most of the fragmentation of DY-12 dye degradation (phenol or another aromatic amine) almost disappear and the concentration of the alkene is very low (ng/l). It is also said that it is well-defined that ozonation reduced notably the contents of the organic synthetic wastewater extracts.

LC-MS/MS mechanistic study

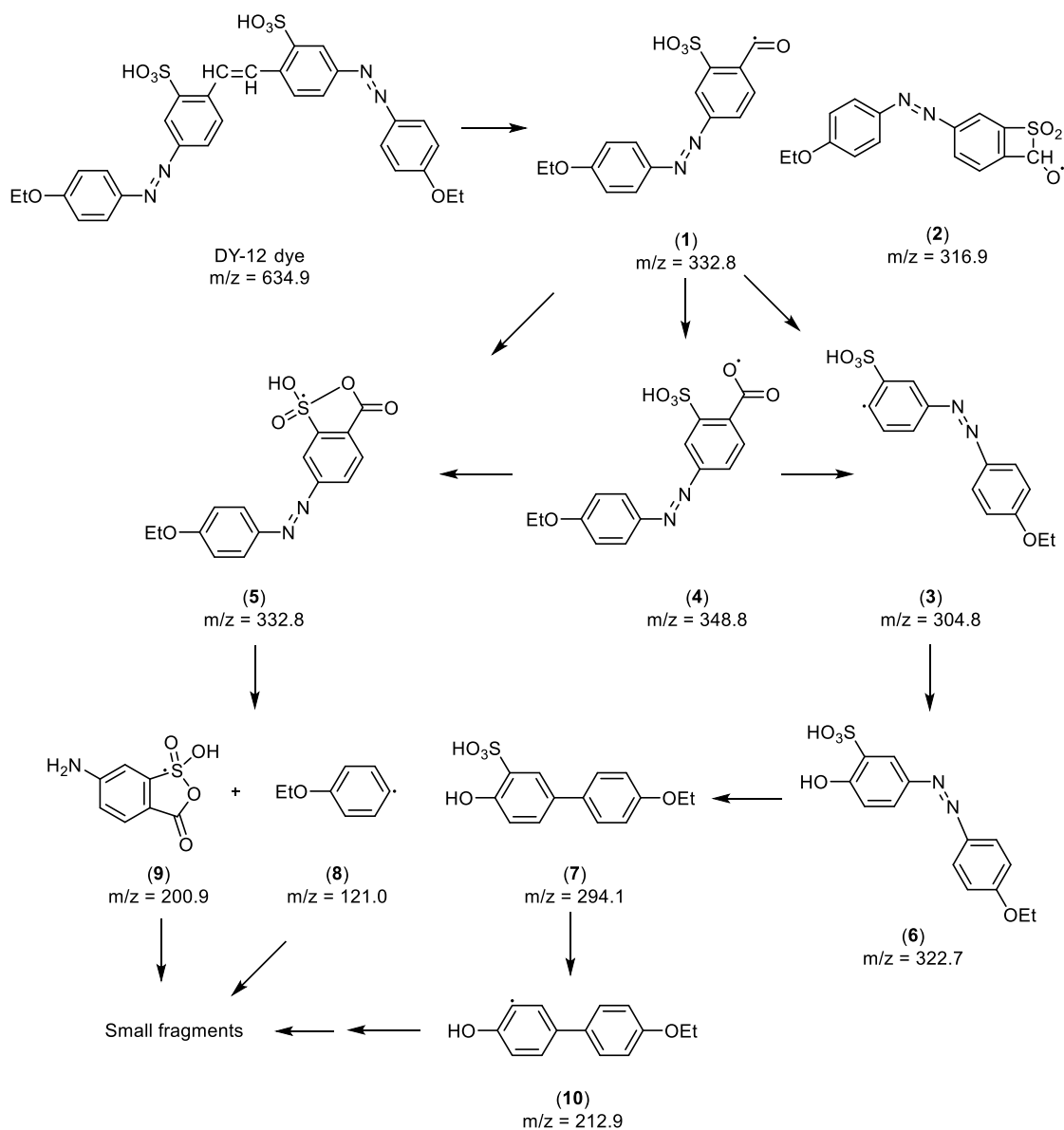
The HPLC-MS/MS analysis of control DY-12 dye showed the presence of one small peak at retention time of 4.213 with m/z 634.9 and 656.9 and one major peak at retention time of 5.211 min have the same two m/z values, which is attributed to the presence of free acid and mono sodium salt of DY-12 dye (Fig. 9). The DY-12 dye decolorization process was analyzed by HPLC-MS/MS at the interval times of 5, 30, 45, 75, 120, 240, 300, 480 and 750 minutes (Fig. 10). After five minutes of treatment the peaks of DY-12 dye were mainly disappeared and three new peaks appeared, small peak of retention time 3.260 min with m/z 303.7 and small peak of retention time 3.946 min with m/z 316.9 and the third peak appeared at retention time 4.544 min with m/z 316.9 and 332.80. After 30 minutes of the treatment many new small peaks were appeared at retention times of 2.097 (m/z 200.90, 278.80 and 322.70), 2.399 (m/z 306.70 and 316.80), 2.567 (348.80), 2.750 (m/z 304.80), 2.997 (m/z 348.80), 3.372 (m/z 332.80), 4.257 (m/z 316.8, 332.8, 348.8) minutes together with the major peak of retention time 4.544 minute of m/z 332.8. The peaks reported after 45 min of treatment were mainly similar to that reported

after 30 min of treatment with only lower peaks intensity while having the same m/z values. The number of peaks reported after 75 min of treatment is increased and the intensity is decreased. However, the major peaks reported belonging mainly to same compounds reported after 30 and 45 minutes of treatment. The monitoring of the DY-12 dye after 750 minutes of treatment showed mainly complete disappear of the above fragments and no aromatic amine or phenol are detected during or at the end of the treatments. The appearance of new minor peaks and disappearance of the major peak in the decolorized dye products elution profile support the biodegradation of DY-12 dye (Fig. 10).

Using this approach, we were able to identify the molecular mass of ten different intermediates produced during DY-12 dye degradation using AOP. The structures of these intermediate were confirmed with Product ions study of MS-MS fragmentation. Scheme 1 shows representative intermediate products of the DY-12 dye degradation. It is also interesting to note that the resulting intermediates produced during DY-12 degradation are aromatic compounds, however, they are quickly degraded to smaller and less-compounds and may eventually be mineralized to CO_2 , H_2SO_4 and ammonium ions.

Toxicity Test

The toxicity rates were evaluated by examining the mortality numbers of Rotifer *B. plicatilis*. The mortality rates in ozone and UV treated 100 mg/l of DY-12 dye was close to 0, and 50% after 0 and 15 minutes, respectively. Whereas the untreated dye of similar concentrations showed 0, and 10%, respectively. So we stop the experiment because the pH of the treated solution was very low after advanced oxidation process about 3.7 so we raise the pH of the treated water to be near the seawater pH (7.50 – 7.60) so the zooplankton can survive and we repeat the experiment and we notice there is no mortality at all in the treated solution even after 1 hour and also no more death after the first 10% of the untreated solution after 15 minutes (Fig. 11). From Fig 11 also, one can see there is no mortality for control samples for the first hour. About 70% of dyes used in the textile industries are azo dyes that identified to be noxious in nature [54]. Furlan et al. [55] reported that, after removing the color content at the end of the treatment process, it also decreased the textile effluent noxiousness generated at the end of the process. The current toxicity test showed that the mortality levels of untreated dye were much more than treated ones. It can be concluded that the treated dyes were less



Scheme 1. HPLC-MS/MS degradation mechanism of DY-12 dye using AOPs

poisonous to the marine aquatic organisms and could be released with less harm to the marine ecosystem. The mortality test revealed obviously that the AOPs had a positive outcome on zooplankton toxicity of the DY-12 dye solution but after the adjustment of the pH of the treated solution.

CONCLUSION

The aim of this study was to investigate the efficiency of applying ozone and the combination of ozone and UV to decolorize and detoxify an aqueous solution of Chrysophenine G (Direct

Yellow 12). From the results obtained, ozone treatment proves to be very effective for complete removal of color and provides more than 85% reduction of COD. The removal efficiency of DY-12 dye color decrease as an increase in initial dye concentration. Faster color removal in caustic media (pH 9) is due to the formation of hydroxyl radicals which have a higher oxidation potential than an O_3 molecule. The enhanced k_d indicated that AOP technique was able to shorten $T_{1/2}$, leading to reduce the reaction time. The oxidation of the DY-12 followed the first-order kinetics with respect to

dye initial concentration. Color removal efficiency was higher in case of UV and O₃ combination as compared to ozone alone and was related to the production of more hydroxyl radicals which more efficient in color removal. Furthermore, GC/MS for identifying the ozonation by-products as well as to evaluate the degradation of the treated aqueous solution showed no toxic compounds. The obtained results also demonstrate that advanced oxidation processes reduced the zooplankton toxicity of the raw solution after adjusting the pH of the treated raw solution to normal seawater pH around 7.50 or 7.60 because, after AOP, the treated solution has pH around 3.7.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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