



## Removal of COD and color from hydrolyzed textile azo dye by combined ozonation and biological treatment

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### ABSTRACT

The application of ozonation has been increasing in recent years, the main disadvantage of this type of treatment being related to the by-products, which can have toxic and carcinogenic properties, and therefore should be studied further. In this study, the combined treatment of ozonation and subsequent biological degradation with a biofilm, to reduce the color and chemical oxygen demand (COD), was investigated. The experimental part of the study consisted of two phases. The first phase was the ozonation process, the results obtained demonstrated that the ozonation of Remazol Black B dye at pH values of 3–11, was effective, partially oxidizing and completely decolorizing the effluent, even at relatively high concentrations of the dye (500 mg/L). Color removal efficiencies greater than 96% were obtained in all cases. The degradation kinetics of ozone is a pseudo-first-order reaction with respect to the dye concentration. It was possible to verify that the ozonation process as a pre-treatment increases the dye degradation efficiency. For the biological treatment, an increase in ozonation time increased the dye concentration reduction in hydrolyzed dye synthetic effluent. The toxicological results of the tests with *Daphnia Magna* showed that there is an increase in toxicity after ozonation and a decrease after submitting the ozonized synthetic wastewater to biological treatment with a biofilm.

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### 1. Introduction

Textile effluents are very difficult to treat satisfactorily due to high variations in their compositions. Strong color is one of the main characteristics of textile effluent and, if not eliminated, it can cause serious problems to the environment. Also, some types of dyes, especially azo dyes and their by-products, may be carcinogenic and mutagenic. The main sources of contamination in textile effluent are the dyeing and finishing processes, because they require the addition of a wide variety of dyes and chemical compounds which are generally organic and have a complex structure.

Due to environmental implications, new technologies have been investigated for degradation of these compounds in textile effluents. Some studies suggest employing advanced oxidative processes (AOPs) in isolation, combined or for preliminary assessment [1–3]. In this study we assessed the efficiency, in terms of color removal and organic matter (in terms of COD) reduction, of a solution of the hydrolyzed dye Remazol Black B. The treatment system

consisted of a combined process of ozonation followed by biological treatment with a biofilm.

There are around 8000 chemicals used as dyes and 10,000 commercial products [4]. The properties of textile dyes, such as brightness, visible color at low concentrations, chemical structure, and resistance to light and chemical attack, make them fairly resistant to microbial degradation [5]. There are numerous chemical structures of dyes and a detailed classification is given in Color Index (CI), a publication which separates dyes into 25 structural classes, most notable of which are the azo dye classes. Azo dyes are comprised of an –N=N– group, and when attached to molecules they become monoazo, diazo or polyazo dyes. Azo dyes currently represent around 60% of the world's market for dyes and are widely used in the dyeing of textile fibers [4].

Large quantities of azo dyes remain in the effluent after finishing the dyeing process. Due to their toxicity and slow degradation, the azo dyes are classified as environmentally hazardous materials. Azo dyes are resistant to biodegradation under aerobic conditions, however, anaerobic treatment can be implemented satisfactorily. The decolorization of azo dyes invariably begins by reductive cleavage of the azo linkage under anaerobic conditions. This leads to the formation of aromatic amines, which may be more toxic than the dye molecules themselves. However, the aromatic amines are easily mineralized under aerobic conditions [6].

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Numerous physico-chemical treatments, including coagulation, flocculation, filtration and membrane adsorption, are used to remove color from textile effluents. It has been found that these treatments have the disadvantages of generating sludge, difficult regeneration of adsorbents and fouling of the membrane [2].

It is necessary to develop appropriate technologies for the control of toxic dyes depending on their particular character. In this regard, new technologies for water and wastewater treatment that destroy or immobilize toxic compounds have been directed toward development strategies, where the treatment of contaminants is the most effective and economical as possible.

Advanced oxidative processes (AOPs) are based on the generation of hydroxyl radicals ( $\text{OH}^-$ ), which have high oxidation potential ( $E^\circ \cong 2.80 \text{ V}$ ) and can promote degradation of a wide range of contaminants in minutes. Hydroxyl radicals can be generated by reactions involving strong oxidizers such as ozone ( $\text{O}_3$ ), hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), the semiconductors titanium dioxide ( $\text{TiO}_2$ ) and zinc oxide ( $\text{ZnO}$ ), and ultraviolet (UV) light. Some oxidative processes have the great advantage of not generating by-products or solids (sludge) and do not involve phase transfer of contaminants (e.g. adsorption on activated carbon) [7]. The final products are carbon dioxide and water. As an example, we can cite the use of hydrogen peroxide and ozone. The degradation of reactive dyes using these processes is an efficient and appropriate chemical method for the treatment of textile effluents, for which decolorization is a major concern [8].

Ozonation is an oxidative process in which the oxidizing agent used is ozone ( $\text{O}_3$ ). Interest in the use of ozone in wastewater treatment has increased considerably in recent years due to the numerous advantages of this process. Amongst them are the high oxidation potential of ozone, even at low concentrations, its high efficiency in the decomposition of organic matter, the addition of oxygen to water, and its low sensitivity to changes in temperature. Although, the cost of ozone production has decreased in recent years, the ozonation process still has a high cost [8]. Reactions involved in ozonation can occur directly between ozone and the organic compounds present in effluent or indirectly through  $\text{OH}^-$  radicals [9,10].

Results presented by Marmagne and Coste [11], show that ozone degrades all kinds of dyes except insoluble disperse dyes and vat dyes, as it reacts slowly and requires more time for degradation. Furthermore, it has been reported that the removal of color in wastewater using ozonation textiles depends on the dye concentration [12–14].

Oxidation of some dyes by ozone has shown that degradation of the olefinic bond results in azobenzene and carbonyl compounds. The azoic  $-\text{N}=\text{N}-$  bond is less reactive than the double bond  $>\text{C}=\text{C}<$ . Muthukumar et al. [15] observed that the structure of azo dyes influences the kinetics of their reaction with ozone, because dyes with naphthalene-rings are oxidized more rapidly when compared to those containing benzene rings. Moreover, they established that dye reactivity with ozone is related to the size of the group. Thus, the order of reactivity for the following compounds is: benzene  $<$  naphthalene  $<$  phenanthrene  $<$  pyrene  $<$  anthracene.

Mehmet and Hasan [13], Konsowa [14], and Azbar et al. [16] have reported that dye oxidation reactions increased slightly with an increase in the solution pH. The greater removal of color that occurs at alkaline pH is due to a greater decomposition of ozone favored by formation of hydroxyl radicals at high pH values. The reaction must be at least pH 7 to improve the ozone decomposition and color removal. Arslan and Isil [17] concluded that the greatest simulated color removal from reactive dye effluent occurred at pH 7.

Effluent treatment may require a combination of processes to obtain satisfactory results, at reasonable cost. Textile wastewater treatment using continuous combined processes of chemical

coagulation, chemical oxidation, electrochemical oxidation, and treatment with activated sludge was investigated by Lin and Peng [18]. For these processes, the quality of the textile effluent treated, in terms of chemical oxygen demand (COD) and transparency, largely exceeded the discharge standards. A combination of chemical and biological processes for textile effluent treatments is of interest due to the chromophore groups of the dyes, which are recalcitrant to biological treatment [19]. Moraes [20] studied the degradation and toxicity reduction of textile effluent combining the photocatalytic process with ozonation. Results showed reductions of 95% for color, 60% for total organic carbon (TOC) and 50% for toxicity (using *Escherichia coli* as the test organism) of the effluent.

According to Mehmet and Hasan [13], ozonation ( $30 \text{ mg/cm}^3$ ) increases the rate of biodegradability of industrial wastewater by a factor of 1.6. Jianguo and Tingwei [21] reported 11–66-fold to increase in the degradation rate for wastewater containing azo dyes, while the increase reached 80-fold for wastewater containing reactive dyes such as Reactive Yellow 84 [22]. These results show that the increase in the degradation rate was influenced by the type of dye and its concentration. Nonetheless, Gianluca and Nicola [23] reported that color removal from textile wastewater treated biologically was dependent on the initial COD value of the textile wastewater.

The main objective of this study is on a removal of COD and color of hydrolyzed textile azo dye (Fig. 1) by combined ozonation and biological treatment. The first stage examined the efficiency of removal by ozonation process and the second evaluated the efficiency by biological process.

## 2. Materials and methods

Considering its wide application in the textile industry, the textile dye Remazol Black B was selected for the ozonation treatment.

For the development of this research Remazol Black B dye (Color Index 20505) was used, with an azo functional group, maximum absorbance of 600 nm and molecular mass of 991 g/mol. Fig. 1 shows the chemical structure of the dye used in this study.

A stock solution of the hydrolyzed dye was prepared according to the methodology presented by Lourenço et al. [24] dissolving 10 g of dye in 1 L of distilled water and adjusting the pH of the solution to 12 with a solution of NaOH 2N. Immediately, the solution was stirred for 1 h at  $80^\circ\text{C}$ , in order to ensure complete hydrolysis of the dye. The dye concentrations used in the ozonation process were obtained from dilution of the stock solution.

### 2.1. Experimental procedure of ozonation stage

The ozone was generated by a Trailgaz-type ozone generator, model-LABO 6LO. This generator operates based on a corona discharge producing ozone from air or pure oxygen. Oxygen was fed to the ozonator with a regulated pressure in the cylinder of  $6 \text{ kg f/cm}^2$ , before entering the ozone generator cell. The pressure was adjusted to  $0.5 \text{ kg f/cm}^2$  using the pressure regulator of the ozonator. The power intensity regulator was set to 50W, as indicated on the wattmeter. Leaving the ozonator, the ozonized gas was circulated through an OMEL-type rotameter and the flow rate was 1 L/min. The equipment was cooled through the continuous circulation of tap water.

Dye solutions were ozonized at room temperature, using a cylindrical glass reactor with an internal diameter of 5.8 cm and height of 1.35 m. The volume of synthetic wastewater used was 5 L, which was recirculated in countercurrent flow (approximately 4 L/min) using a centrifugal pump, and the gas feed was injected continuously through a porous diffuser which consisted of three porous stones, placed at the bottom of the reactor. The off-gas or a por-

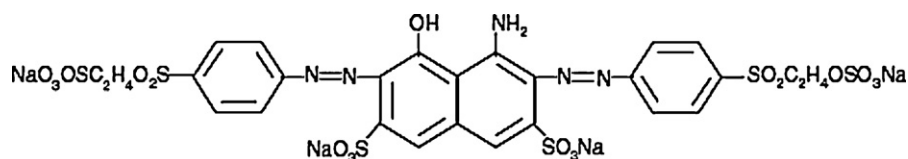


Fig. 1. Chemical structure of the dye Remazol Black B.

tion of gas mixture that was not retained in the liquid mass exited through the top of the column. This column contained two gas scrubber bottles in series to ensure that, occasionally, a small portion of ozone gas, which had not been eliminated in the first contact with potassium iodide solution in the first bottle, was placed in contact with the solution of the second of gas scrubber bottle. As ozone gas is carcinogen, the ozone molecules had to be broken down before the gas was released into the atmosphere.

## 2.2. Experimental procedure for biological treatment stage

Tests were performed in batch mode, on batch scale, using 250 mL Erlenmeyer flasks as bioreactors. Bioreactors were inoculated with biomass from a municipal wastewater treatment plant (CASAN) in Florianópolis, Santa Catarina, Brazil, with an initial concentration of volatile suspended solids (VSS) 10.9 g VSS/L, aerated with an air flow using a porous diffuser and agitated at 130 rpm using a Dubnoff water bath shaker (New Ethics—model 304).

The support material used for immobilization of the biomass was activated carbon particles, provided by Carbomafra, particle size 8–10 mesh, sieved in a system of Brasinox sieves.

To perform the adaptation and immobilization of the biomass on the support, the bioreactors were fed with a culture medium with a composition based on that proposed by Shim and Yang [25]. Sucrose (100 mg/L) was used as a carbon source and the pH of the final solution was 7. The bioreactors were shaken and aerated at room temperature during the whole experiment, in order to mix the medium and avoid concentration gradients within the bioreactor. Dissolved oxygen concentration and the pH in the medium were monitored daily to ensure oxygen concentrations between 6 and 7 mg/L and pH between 6 and 8.

Once the biomass had been adapted and immobilized, the kinetics of the Remazol Black R dye was obtained using a stock solution of ozonized dye. The initial concentration and pH of the dye selected to perform biological treatment was 100, 250 and 500 mg/L, with an initial pH of 9. These solutions were subject to various ozonation times (2–60 min), leaving them for at least 24 h before feeding the biological reactor. Thus, possible interferences from any quantity of dissolved ozone (residual) in the biological treatment were eliminated. The final pH of the stock solution was adjusted to 7, using NaOH or H<sub>2</sub>SO<sub>4</sub>. Samples to obtain the degradation kinetics of the solutions were collected from each reactor at defined time intervals. During the tests each reactor was fed daily with 100 mg/L of sucrose, with constant aeration in order to maintain dissolved oxygen concentrations between 6 and 7 mg O<sub>2</sub>/L.

## 2.3. Analytical methods

The calibration curve was built according to the Lambert–Beer law, i.e. the graph of a solution of a known concentration of dye versus absorbance measured on a spectrophotometer. Thus, using the calibration curve, one can identify the concentration of dye in different solutions of the experimental tests. The absorbance of the samples was measured by the integration method developed by Law et al. [26] and Wu et al. [27], cited by Wu and Wang [28]. This involves scanning the absorbance of samples from 350 to 700 nm, and integrating the area under the absorbance curve. Color analysis

was performed with spectrum scanning in the wavelength range of 190–700 nm, using a Shimadzu UV mini 1240 spectrophotometer.

Residual ozone concentration of the reactor was determined at previously selected intervals during ozonation of this solution using the indigo colorimetric method [29]. The amount of ozone produced and consumed was determined by the iodometric method described in the Standard Methods for Examination of Water and Wastewater [29]. Measurement of COD was performed according to the procedure described in Standard Methods for the Examination of Water and Wastewater [29].

Acute toxicity tests were conducted using the microcrustacean *Daphnia magna*. Tests consisted of exposing young *D. magna* to various dilutions of the test agent for a period of 48 h, where acute toxic effect was determined by the loss of body motion. The tests were carried out in 25 mL beakers, in duplicate for each concentration, in addition to control with the dilution water. Ten young *Daphnia* were placed in each beaker (6–24 h of life). Synthetic effluents, before and after ozonation and biologic treatment, were evaluated at different concentrations. Tests were maintained at 20 ± 2 °C for 48 h in a dark room without food. After 24 and 48 h of incubation, the immobile bodies were counted to determine the toxicity factor using Spearman–Karber software (the 1978 version of the *Trimmed Spearman–Karber* method developed by Montana State University).

In order to verify bacterial attachment on the surface of the support and observe the biofilm structure, micrographs were taken by scanning electron microscope (SEM) at the Laboratory of Materials of Mechanical Engineering Department of the Federal University of Santa Catarina.

The percentage removal of the parameters evaluated (decoloration and degradation) was determined using the following equation:

$$\% \text{ removal} = \left( \frac{C_0 - C}{C_0} \right) \times 100$$

where  $C_0$  and  $C$  are initial and final concentrations of dye and COD in the synthetic wastewater.

## 3. Results and discussion

### 3.1. Effect of pH

In Fig. 2 it can be observed that the initial pH values of 5, 7, 9 and 11 decrease with ozonation time initial dye concentration of 500 mg/L, until a steady state is reached. This indicates the generation of by-products of acidic nature (inorganic acids and organic anions), as a result of oxidation by ozone. It can be noted that with an initial pH of 3 there is a slight pH increase until relatively stable values are reached, close to those observed for the other initial pH values.

The reaction of ozone both with hydroxyl anions and the products of degradation formed by oxidation via hydroxyl radicals (usually carboxylic acids) tends to decrease the pH of the solution during treatment. The decrease in pH is very pronounced in the initial phase of the process (particularly for alkaline solutions) because there is a greater ozone consumption and dye degradation/mineralization.

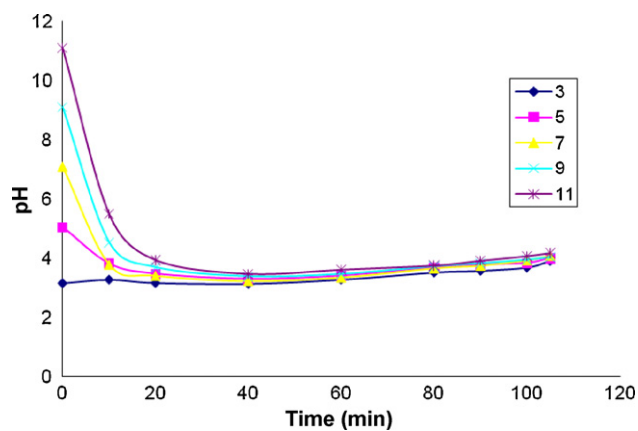


Fig. 2. Variation in pH with ozonation time for initial dye concentration of 500 mg/L.

Fig. 3 shows the results for the reduction in the dye concentration during ozonation of a Remazol Black B dye solution with an initial concentration of 500 mg/L, with different initial pH values.

Alvares et al. [30], Soares et al. [31] and several other researchers have observed that ozone decomposition is directly affected by the solution pH. Hydroxyl radicals are formed from ozone decomposition at high pH values, while molecular ozone remains as the main oxidant at low pH. The degree of decolorization is favored by direct ozone attack, at low pH, since molecular ozone selectively attacks chromophore groups. Hydroxyl radicals have a greater oxidative power and are less selective than molecular ozone, leading to a decrease in decolorization and an increase in mineralization at higher pH.

Through analysis of Fig. 4, we can infer that there was no significant effect of pH on dye removal. As mentioned earlier, several researchers have observed that dye reduction is dependent on the solution pH. However, this study demonstrates that the reduction in the dye concentration was independent of the initial pH value in the range of 3–11. This is probably due to some researchers using buffer solutions to control the pH and others adjusting the initial pH with an acid or base solution, the latter being the procedure adopted herein.

Furthermore, for higher pH values there is a slight increase in the percentage of ozone transfer efficiency, as shown in Fig. 4. This can be attributed to the formation of hydroxyl radicals through an indirect oxidation mechanism involving a series of chain reactions with radical species regeneration routes, which are more effective in terms of mineralization.

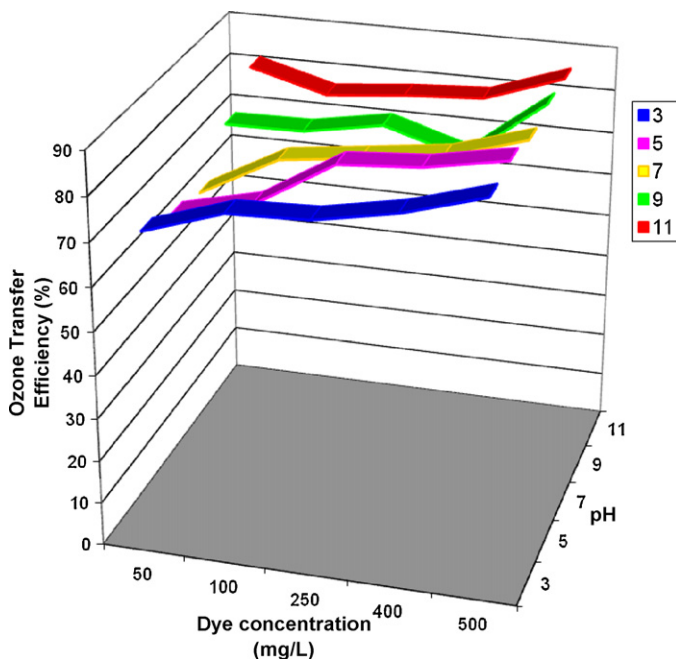


Fig. 4. Variation in ozone transfer with dye concentration and initial pH values.

### 3.2. Effect of ozonation on COD removal

There is a tendency toward a decrease in COD with increasing ozonation time, although in some cases the values increase with increasing ozonation time, especially for times of 100 and 105 min. The results for the COD removals for an initial concentration of 500 mg/L are shown in Fig. 5 and Table 1. The increase in COD may be due to dye molecules being oxidized by ozonation, resulting in small organic molecular fragments, such as acetic acid, aldehydes, ketones, which are not completely mineralized under the oxidative conditions described, contributing to an increase in COD with ozonation time.

According to Wang et al. [32], these small molecules contribute significantly to the COD and cannot be completely removed by ozonation. However, components which contribute to the COD are easily biodegraded in activated sludge processes.

It can be observed through the results presented in Figs. 6 and 7 that as the dye concentration increases, the dye removal decreases, within a certain time period. For example, for an ozonation time of

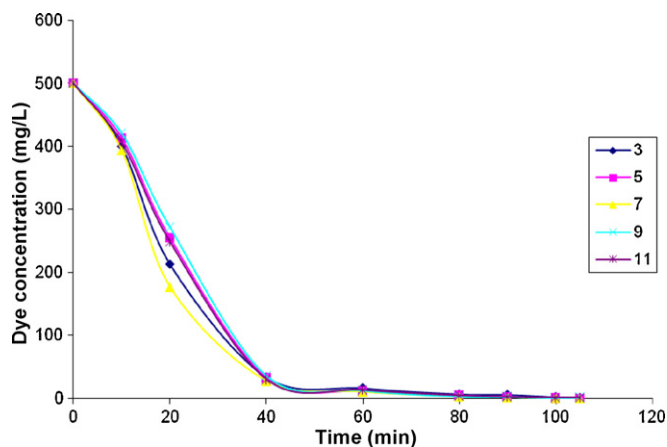


Fig. 3. Reduction in initial dye concentration of 500 mg/L at different pH values.

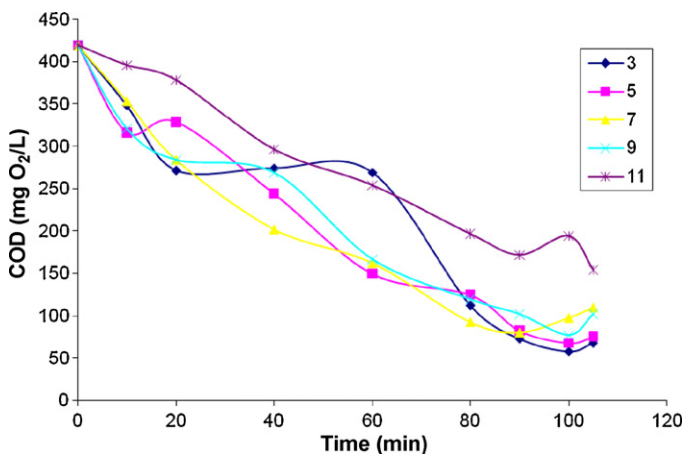
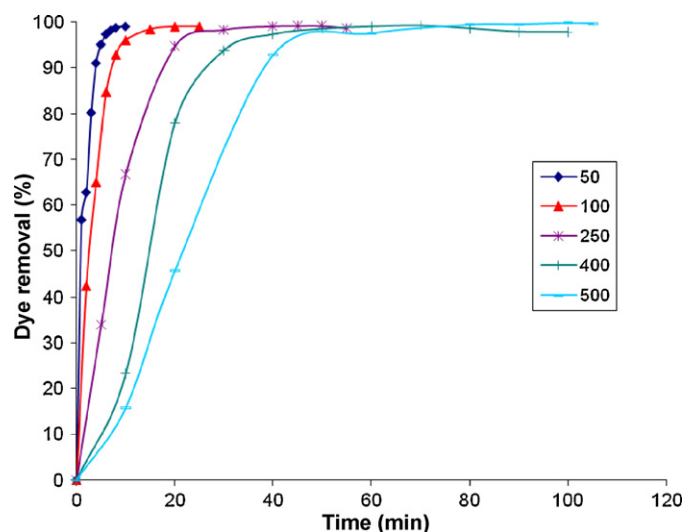


Fig. 5. COD removal in relation to ozonation time for different pH values with initial dye concentration of 500 mg/L.

**Table 1**  
COD values in relation to ozonation time for different pH values.

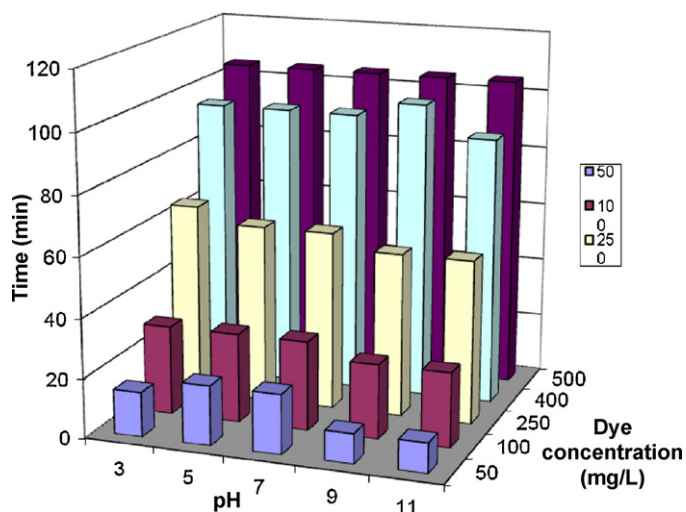
Time (min)	pH				
	3	5	7	9	11
0	419	419	419	419	419
10	348	316	353	321	396
20	271	328	284	284	378
40	274	244	202	269	296
60	269	149	162	167	254
80	112	124	92	119	197
90	72	82	80	102	172
100	57	67	97	77	194
105	67	75	109	102	154

Initial dye concentration: 500 mg/L.

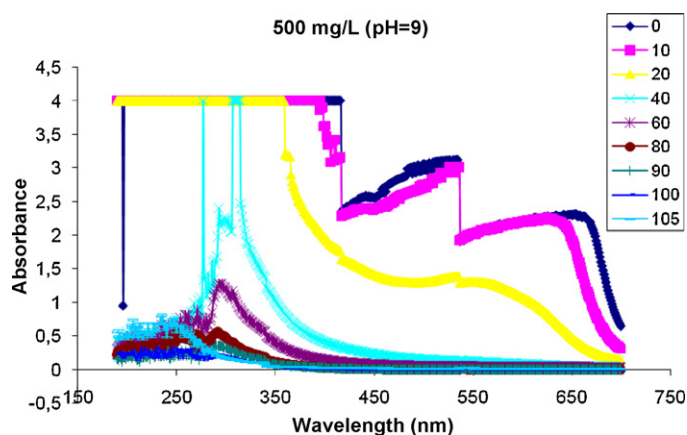


**Fig. 6.** Color removal with ozonation time for different initial dye concentrations.

10 min, the dye removal percentages were 99.1; 96.1; 66.8; 23.3 and 15.8% for initial dye concentrations of 50, 100, 250, 400 and 500 mg/L, respectively. There is a reduction of over 80% for a dye concentration of 500 mg/L compared with 50 mg/L. In general, the color removals obtained were greater than 96% for the different dye concentrations and initial pH values, which verifies the ozonation efficiency in terms of color removal.



**Fig. 7.** Variation in ozonation time with initial pH and dye concentration.



**Fig. 8.** Evolution of hydrolyzed Remazol Black B dye absorbance during ozonation.

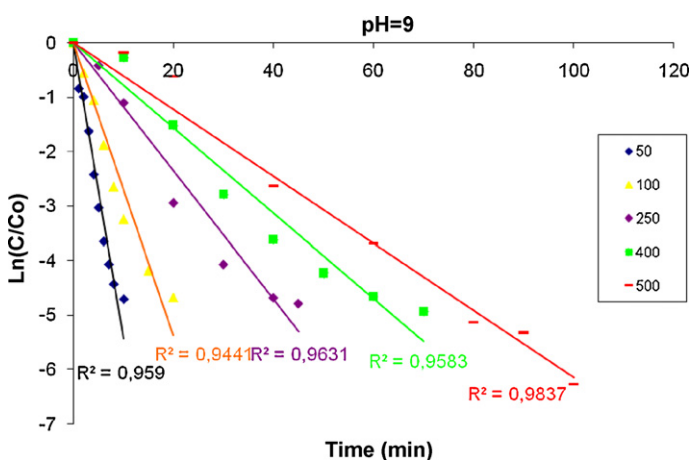
Fig. 7 shows that for higher dye concentrations it is necessary a longer ozonation time to degrade the dye molecule. There is also a slight increase in the ozonation time required as the pH values decrease.

In all cases the absorbance reduces progressively with ozonation and there is no formation of new peaks. A reduction in absorbance in the visible spectrum shows dye decolorization through cleavage of the chromophoric group, which in azo dyes is characterized by the  $-N=N-$  bond, as shown in Fig. 8. According to Alvares et al. [30], the cleavage of the chromophoric group suggests that color removal is the first step of partial oxidation.

In the non-visible region, there is also an absorbance decrease, which indicates the formation of new compounds or a breaking of these molecules into simpler ones. During this process, more complex non-biodegradable compounds are converted to simpler products that contain in their chemical structure an increased percentage of oxygen in the form of hydroxyl, carboxyl or aldehyde functional groups.

In the literature, it is reported that dye ozonation is a first-order kinetics reaction, with respect to ozone and dye concentration [33]. Therefore, the ozonation rate increases with both ozone and dye concentration. However, in this study, the ozone concentration injected is a constant, making the degradation kinetics a pseudo-first-order reaction with respect to the dye concentration.

Thus, as shown in Fig. 9, ozonation of the synthetic wastewater of the hydrolyzed dye follows a pseudo-first-order kinetics reaction with respect to dye concentration, where the decolorization rate decreased with increasing dye concentration.



**Fig. 9.** Pseudo-first-order degradation kinetics of hydrolyzed Remazol Black B dye.

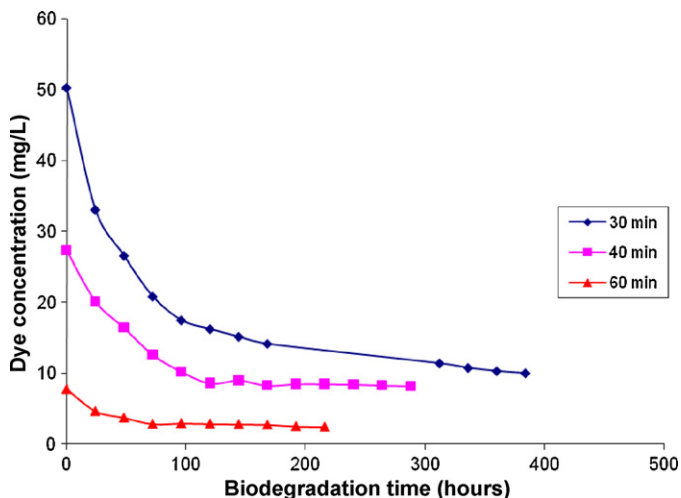


Fig. 10. Biodegradation of synthetic wastewater of hydrolyzed dye for different ozonation times. Initial dye concentration: 500 mg/L.

### 3.3. Effect of initial dye concentration

The dye removal was similar for the three initial dye concentrations of 100, 250 and 500 mg/L, where the formation of intermediates in the first few minutes of the ozonation step leads to a lower dye removal efficiency, as shown in Fig. 10.

For a longer ozonation times there is a greater reduction in the synthetic wastewater dye concentration through biological treatment. Considering the dye reduction, it was noted that for longer ozonation times (60 min for an initial concentration of 500 mg/L), final dye concentrations of approximately 2 mg/L are obtained for all cases, while for a shorter ozonation times concentrations reach levels between 6 and 10 mg/L.

Fig. 11 shows the profiles for the biodegradation of ozonized and non-ozonized hydrolyzed dye solution employing biofilm adaptation for the two different wastewaters.

It can be verified that ozonation as a pre-treatment process increases the dye biodegradation efficiency, since for a degradation time of 360 h, dye removal efficiencies of 91, 59.3 and 79.3% were obtained for ozonized, non-ozonized hydrolyzed dye solution (1) and hydrolyzed dye solution (2), respectively.

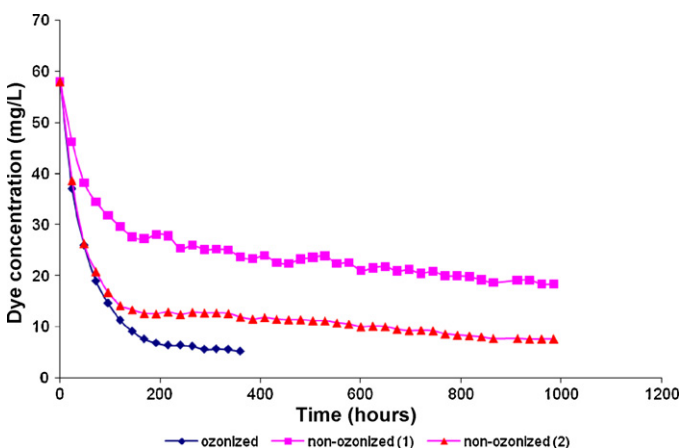


Fig. 11. Comparison of biodegradation profiles of ozonized and non-ozonized hydrolyzed dye solution (ozonation time: 10 min). (a) Non-ozonized hydrolyzed dye solution (1): biodegraded with biomass adapted with ozonized hydrolyzed dye solution. (b) Non-ozonized hydrolyzed dye solution (2): biodegraded with biomass adapted with non-ozonized hydrolyzed dye solution.

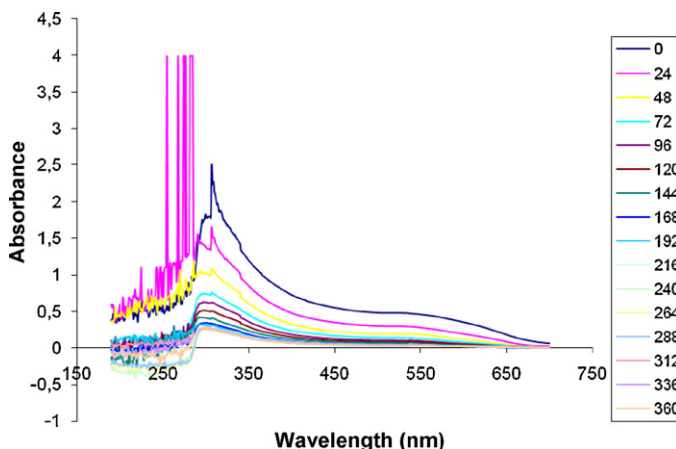


Fig. 12. Variation in absorbance during biological process using biofilm with ozonized hydrolyzed Remazol Black B dye. Initial concentration: 250 mg/L, ozonation time: 10 min.

From the results obtained in the experiments it was found that for 144 h of biodegradation dye removal efficiencies of 52 and 77% were achieved for the non-ozonized hydrolyzed dye solutions (1) and (2), respectively, reaching a stationary phase where there is a significant increase in the removal efficiency.

Differences between the removal efficiencies of the non-ozonized hydrolyzed dye solutions (1) and (2) are due to the fact that the biomass used for the treatment was adapted to different solutions. In biomass submitted to adaptation with non-ozonized hydrolyzed dye solution the development of microorganisms is inhibited, in contrast to biomass adapted to ozonized hydrolyzed dye solution. The latter has a greater capacity to withstand the presence of more toxic compounds than the former.

According to the results shown in the dye degradation profiles of Fig. 11, biological treatment alone is not sufficient to reduce dye concentrations in textile effluent treatment.

For all of the biodegradation tests, with the synthetic wastewater submitted to different ozonation times, an absorbance decrease in the non-visible wavelength range can be observed, as shown in Fig. 12. This is due to the fact that the biological process is more efficient at mineralizing the by-products of the ozonation, but less efficient at color reduction than the ozonation process itself.

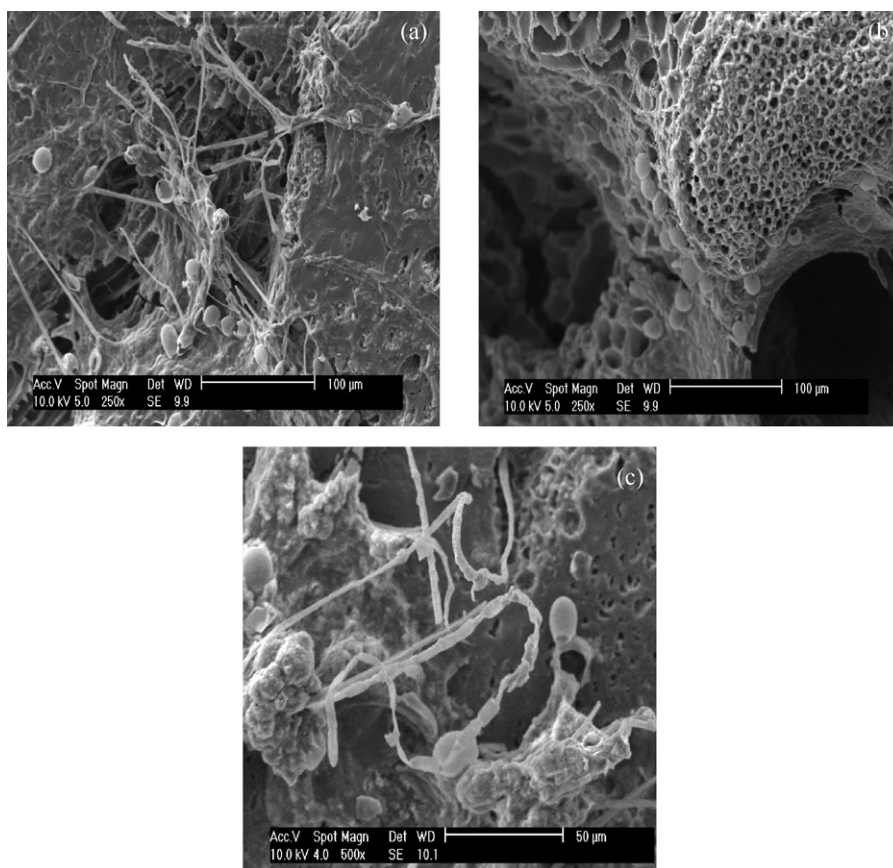
### 3.4. Analysis of biofilm micrographs

Fig. 13 shows the carbon surface containing the biofilm with microorganisms adapted to synthetic wastewater. Micrographs show the presence of microorganisms (spherical shape with around 18.6 μm diameter) in the open carbon pores.

In the microscopic observations of the biofilm the following were identified:

- Presence of filaments and bacteria in biofilms formed initially without being submitted to any adaptation.
- Presence of bacteria or protozoa in biofilm formed after adaptation to a non-ozonized hydrolyzed dye solution.
- Abundance of filaments in biofilm formed after adaptation to ozonized hydrolyzed dye solution. Presence of lower quantity of bacteria when compared with biofilm adapted to non-ozonized hydrolyzed dye solution.

The best performance for color removal, as seen in Fig. 12, could be attributed to the presence of filamentous microorganisms in the biofilms adapted to ozonized hydrolyzed dye solution, since the biofilm adapted to non-ozonized hydrolyzed dye solution inhibited



**Fig. 13.** Micrographs of biofilms formed on support under different conditions. (a) Biofilm without adaptation (magnification of 250 $\times$ ); (b) biofilm after adaptation with non-ozonized hydrolyzed dye solution (magnification of 250 $\times$ ); (c) biofilm after adaptation with ozonized hydrolyzed dye solution (magnification of 500 $\times$ ).

ited the formation of microorganisms, decreasing the color removal efficiencies.

In many cases, filamentous forms may emerge in the development of a biofilm, and *Hyphomicrobium*, *Sphaerotilus* and *Beggiatoa* are often identified. With a lack of substrate, these forms can provide an ecological advantage due to the fact that their surface/volume ratio allows the flow of the liquid to better extract the substrate [34].

### 3.5. Analysis of toxicity

From the results shown in Table 2, it can be verified that the toxicity of synthetic wastewater after ozonation tended to increase (decrease in the  $EC_{50}$  values), whereas the toxicity of the synthetic wastewater after biological treatment tended to decrease (increase  $EC_{50}$  values). The low  $EC_{50}$  values evaluated in the tests with ozonized synthetic wastewater show the presence of high toxicity for all samples.

The results of this study are in agreement with those of two acute toxicity tests reported in the literature: the bioluminescence test (*Vibrio fischer* II) and the neutral red cytotoxicity test [34], where partial oxidation of Remazol Black 5 dye solutions with ozone occurred within the first 150 min of a total of 360 min of oxidation, leading to the formation of by-products with high toxic potential. The results reported herein are also consistent with those obtained in tests using respirometry [8], which showed that for both Orange II and Acid Red 27 ozonation produces intermediates that can inhibit the growth of degradation microorganism present in tests used to determine BOD.

**Table 2**

Results of toxicity tests with *Daphnia magna*.

Sample	pH	$EC_{50}$	DF
Synthetic wastewater of hydrolyzed dye not submitted to treatment			
100 mg/L	6.10	46	16
250 mg/L	6.03	20	16
500 mg/L	5.52	16	16
Synthetic wastewater ozonized			
100 mg/L, $t = 2$ min	4.98	Very toxic	>128
100 mg/L, $t = 4$ min	3.73	45	32
100 mg/L, $t = 6$ min	6.37	NC	>32
250 mg/L, $t = 10$ min	3.20	6	32
250 mg/L, $t = 15$ min	4.88	62	8
250 mg/L, $t = 20$ min	2.99	9	32
500 mg/L, $t = 30$ min	3.27	13	32
500 mg/L, $t = 40$ min	3.01	10	16
500 mg/L, $t = 60$ min	3.85	Very toxic	>128
Synthetic wastewater ozonized after biological treatment			
100 mg/L, $t = 2$ min	7.06	19	>16
100 mg/L, $t = 4$ min	7.20	14	>8
100 mg/L, $t = 6$ min	6.40	T.E.	8
250 mg/L, $t = 10$ min	6.07	33	8
250 mg/L, $t = 15$ min	6.05	Non-toxic	1
250 mg/L, $t = 20$ min	6.62	NC	8
500 mg/L, $t = 30$ min	7.97	T.E.	8
500 mg/L, $t = 40$ min	7.02	T.E.	4
500 mg/L, $t = 60$ min	6.64	Non-toxic	1

$t$ : ozonation time (min); DF: dilution factor; NC: not calculable; TE: toxicity evident.

## 4. Conclusions

It is concluded from this study that initial pH values ranging from 3 to 11 do not have a marked effect on dye removal efficiency

in the ozonation process. It is worth mentioning that in this study a buffer solution was not used to maintain the pH constant.

The initial pH values decreased with ozonation time, achieving a steady state, which is indicative of the generation of by-products with acidic nature (inorganic anions and organic acids) as a result of partial oxidation by ozone.

There is a trend toward a decrease in COD with increased ozonation time, although occasional increases of COD were observed in the ozonation process due to dye molecules being oxidized resulting in small organic molecular fragments, such as acetic acid, aldehydes, ketones, contributing to the increase in COD during ozonation.

Ozonation as a pre-treatment for combined chemical-biological treatment, is a potential process for enhanced color removal and biodegradability of wastewaters containing azo dyes such as Remazol Black B, once the appropriate ozonation period is determined. In this context, pre-treatment with ozone is economically desirable due to the lower quantity of ozone required for the mineralization of compounds, and the subsequent biological process will complete the mineralization with lower operational costs than those obtained with chemical processes.

Furthermore, acute toxicity tests with *Daphnia magna* showed that pre-treatment with ozonation generates more toxic effluents when compared with the initial synthetic wastewater. For this reason it is necessary to submit the effluent to posterior processes to reduce the toxicity. Thus, the integrated process of pre-ozonation and subsequent biological treatment of biodegradable products is presented as an efficient and economical combined process, since biological treatment with a biofilm to complete the mineralization of compounds in some cases reduces or eliminates the toxicity of the ozonized effluent.

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