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Effects of dye additives on the ozonation process and oxidation by-products: a comparative study using hydrolyzed C.I. *Reactive Red 120*

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Abstract

Decolorization of azo reactive dyes by ozone has been described by several authors. However, the efficiency of ozonation and the by-products produced by dye additives/impurities such as synthetic precursors, by-products, salts and dispersing agents in commercial azo dyes during the oxidative treatment have not been reported. To investigate the effects of such impurities on the ozonation process and on the formation of oxidation by-products, a commercially available azo reactive dye C.I. *Reactive Red 120* was chosen as a model compound. Experiments were conducted in aqueous solutions of hydrolyzed C.I. *Reactive Red 120* (200 mg/l) either with or without purification. Treatment efficiencies of purified and unpurified dye were evaluated in terms of COD, BOD_{28}, absorbance and initial decolorization rates. Additionally, organic and inorganic anions were analyzed by HPIC. After 28 days of incubation the BOD_{28}/COD ratio increased from 0.22 to 0.48 and from 0.05 to 0.26 for purified and unpurified C.I. *Reactive Red 120*, respectively, indicating an enhancement of biodegradable compounds in the ozonated solutions. The results also illustrate that the dye additives/impurities in commercial dyes affect the microbial activity as well as the biodegradability. It is therefore recommended that dyes should be purified before ozonation, if detailed information on the degradation processes and the resulting oxidation by-products are required.

Keywords: Purification; Ozonation; C. I. *Reactive Red 120*; Azo Dye; Textile dyeing and finishing industry

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1. Introduction

The major limitation in treating textile wastewater containing azo reactive dyes is their resistance to conventional biological treatment processes [1,2]. A significant remainder of about 40–70% of COD and color in textile finishing industry effluents after conventional biological treatment requires an advanced treatment for improving the quality [3–6]. Commercial azo dyes used are mixtures of dyes including other additives such as intermediates produced during dye

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synthesis (e.g. naphthaline–sulfonic acids) as well as aromatic derivatives are suspected to be toxic, while other non-toxic products such as sodium chloride and sodium sulfate [7–9]. However, at present the information available is scarce concerning the role of these dye impurities on the formation of toxic or recalcitrant by-products and on biodegradability after oxidative treatment.

The application of ozone as a pretreatment for the improvement of wastewater biodegradability is an emerging process. It has been shown that ozone cleaves the conjugated bonds of azo dye chromophores, leading to color removal and enhancing biodegradability. An increase in the ratio of BOD/COD indicates an improved biodegradability of recalcitrant substrates [10–14]. Since long-term ozonation is expensive, a decrease of the ozonation time and amount of ozone used would help to reduce treatment costs. Partial ozonation as a pretreatment step could be an alternative whereas its major disadvantage of generation of toxic by-products and incomplete mineralization has to be considered. A further risk of ozone pretreatment is that previously biodegradable compounds may form recalcitrant by-products that inhibit biomass activity [15–18]. Previous studies [19] confirmed that short-term ozonation of a model aqueous azo dye solution (C.I. *Reactive Black 5*) and of dye-bath effluents from a textile dyeing and finishing industry formed toxic by-products. It could be shown that partial oxidation of biodegradable matter formed recalcitrant compounds as confirmed by the results of the biodegradability and bioluminescence tests [19,20].

Hence, the main objectives of the present study is to investigate the effect of commercial azo reactive dye C.I. *Reactive Red 120* and its additives/impurities on the formation of potential toxic by-products and their inhibitory effect on the microbial activity during short- and long-term ozonation.

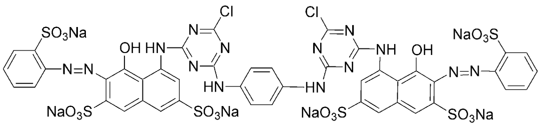

Fig. 1. Characteristics of *C.I. Reactive Red 120*. Molecular formula: $C_{44}H_{24}Cl_2N_{14}Na_6O_{20}S_6$ (as sodium salts); Mw: 1469.98 g/mol; water solubility: 70 g/l.

2. Material and methods

The azo reactive dye C.I. *Reactive Red 120* used for the experiments was obtained from BASF (Germany), as a commercially available dye with a formulation designated as Procion Red H-E3B, of 75% purity. The main characteristics are shown in Fig. 1.

Since the efficiency of ozonation might be affected by the existence of dye additives/impurities the dye was purified to over 90% calculated using UV/VIS according to 75% purity of original commercial dye employing the well known *N*,*N*-dimethyl formamide acetone method [21]. To simulate dye-bath effluents from dyeing processes with azo reactive dyes, hydrolysis of the dye was accomplished by dissolving it in ultra-pure water, adjusting the pH to 10 with 1 mol/l NaOH and boiling for 3 h (95 °C) under reflux. All solutions used in experiments were prepared using-ultra pure water (18.2 MΩ•cm).

Ozone was generated from dried air by an ozone generator Ozon Erzeuger 24 g (*Erwin Sander Elektroapparatebau*). A detailed description of the equipment and operation procedures is described in previous work [2,11].

In this study, two different ozone concentrations were used, namely, a rather low concentration of ozone (8.9 mg/l) to carry out chemical oxidation to facilitate analysis of the initial by-products and an ozone concentration of 20.0 mg/l for biodegradation (BOD_{28}) experiments. For the analysis of breakdown products, samples of 30 ml were collected at defined time intervals and were flushed by N_2 for 30 min in order to remove the residual ozone gas and were filtered with 0.1 μm membrane filter. Decolorization efficiency of ozonation process was determined by measurements of absorbance at wavelengths ranging from 190 to 600 nm by a *Varian Cary 1* spectrophotometer. The maximum visible λ_{max} (535 nm) was employed as a base for characterization of color reduction percentage. COD was determined with commercially available test kits (Machery & Nagel) for dye solutions without significant chloride content (< 1 g/l).

Biodegradability of the aqueous dye samples was determined with a Voith Sapromat, Typ D throughout an incubation period of 28 days. The oxygen consumption was measured according to DIN L22 and DIN EN ISO 29408 [19, 22]. Previous biodegradation experiments (BOD_{28}) with a respirometer indicated that dye concentrations lower than 200 mg/l were too low to exhibit rational oxygen consumption for the incubation period of 28 days [19]. Therefore, triplicates of dye solutions at higher concentrations (500 mg/l) were subjected to further biodegradation after 0, 30, 60, 90 and 150 min of ozonation (20.0 mg O_3/l). The results of the experiments are presented in terms of the mean oxygen consumption of these three measurements as a function of degradation time.

Inorganic and organic anions were determined by high performance ion chromatography (HPIC, Dionex 500) equipped with an IonPac AS 11 column (Dionex) and operated in suppressed conductivity detection mode. Samples of 50 μl injected by an automatic sampler were eluted by a NaOH/water gradient at a flow rate of 1.5 ml min^{-1}.

3. Results and discussion

In Tables 1 and 2, the results of conventional parameters of the aqueous solution of purified and unpurified hydrolyzed *C.I. Reactive Red 120* (200 mg/l) prior to and after various times of ozonation are summarized.

The absorbance (A/A_0) of aqueous solutions of hydrolyzed pure and unpurified dye measured at different intervals of ozonation shows the disappearance of the absorption band at 535 nm with increasing time. Decolorization was almost complete after 150 min of ozonation. Several authors

Table 1
Conventional parameters of ozonation results of purified hydrolyzed *C.I. Reactive Red 120* (C_{dye} = 200 mg/l; ozone concentration 8.9 mg/l)

T (min)	$A\ \lambda_{max}$ 535 nm (AU)	$A/A_0\ \lambda_{max}$ 535 nm (%)	pH at 21 °C	Conductivity at 21 °C (μS/cm)	COD (mg O_2/l)	COD/COD_0 (%)
0	0.5689	100.0	10	94	178	100.0
10	0.5468	96.1	6.30	125	168	94.9
20	0.3316	58.2	4.42	113	151	84.8
30	0.2668	46.9	3.96	140	145	81.5
45	0.1821	32.0	3.62	174	145	81.5
60	0.1136	20.0	3.46	209	138	77.5
90	0.0357	6.27	3.22	274	132	74.1
120	0.0053	0.93	3.08	354	114	64.0
150	0.0003	0.05	2.93	456	98	55.0

Table 2
Conventional parameters of ozonation results of unpurified hydrolyzed *C.I. Reactive Red 120* (C_{dye} = 200 mg/l; ozone concentration 8.9 mg/l)

T (min)	$A\ \lambda_{max}$ 535 nm (AU)	$A/A_0\ \lambda_{max}$ 535 nm (%)	pH at 21 °C	Conductivity at 21 °C (μS/cm)	COD (mg O_2/l)	COD/COD_0 (%)
0	0.4764	100.0	10	166	173	100.0
10	0.2979	62.5	5.83	294	150	86.7
20	0.2189	45.9	4.41	169	144	83.2
30	0.1627	34.1	3.98	190	140	80.9
45	0.1094	23.0	3.73	215	138	79.7
60	0.0656	13.8	3.52	246	124	71.7
90	0.0199	4.2	3.32	308	109	63.0
120	0.0024	0.5	3.16	366	99	57.2
150	0.0000	0.0	3.02	420	90	52.0

reported that the decolorization of dyes by ozone followed first order reaction kinetics with respect to both ozone and dye concentrations [21, 23]. The corresponding first-order correlation is shown in Fig. 2. The reaction rate constant, k, for unpurified *C.I. Reactive Red 120* ($0.0392\ \text{min}^{-1}$) was found to be higher than that of purified *C.I. Reactive Red 120* ($0.0336\ \text{min}^{-1}$). The UV absorbance of the initial dye with and without purification (Tables 1 and 2) confirms that the initial dye concentration of purified *C.I. Reactive Red 120* is higher than unpurified dye sample.

As it is shown in Tables 1 and 2, the pH of the solutions dropped within the first 90 min from 10.0 to 3.52–2.93 for both synthetic dye samples and then remained constant, while throughout the process the conductivity increased continuously.

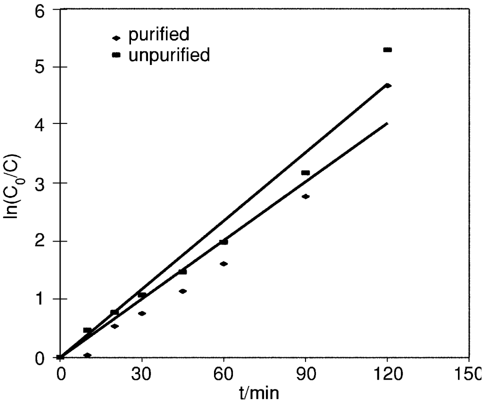

Fig. 2. The pseudo-first order decay rate constants during the ozonation (8.9 mg/l) of purified and unpurified hydrolyzed *C.I. Reactive Red 120* ($C_{\text{dye}} = 200$ mg/l).

Several authors have stated that the pH of water in single-phase ozonation affects O_3 decomposition. When pH increases, the rate of ozone decomposition increases significantly as well. Consequently, the oxidizing agent in acidic solution is ozone, while in basic and neutral solution it is the hydroxyl radical [23–25]. Ozone has an oxidation potential 2.07 V, the hydroxyl radical of 2.80 V. Therefore the decomposition reaction is faster in basic and neutral solutions. As the solution becomes more acidic at prolonged ozonation periods, the organic compounds cannot be oxidized to carbon dioxide but to carboxylic acids [26]. Tables 1 and 2 state that after 150 min ozonation, the COD of both aqueous dye solutions decreased to 54 and 52% of the initial value, indicating the remaining of small organic molecular fragments along with the destruction of the dye

To achieve detailed information on the reaction processes during ozonation and the resulting oxidation end-products organic and inorganic anions were analyzed. Sulfate, nitrate, formate and oxalate were identified as the main oxidation end-products of purified and unpurified dye as shown in Figs. 3 and 4. The concentration of the identified ions increased with the ozonation time. The production of nitrate can be attributed to the oxidation of the amino groups. Oxidation and cleavage of the sulfonate groups from the benzene and

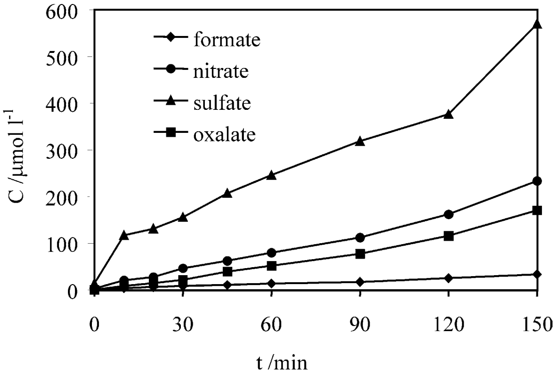

Fig. 3. Concentrations of sulfate, nitrate, formate, oxalate vs the ozonation time (8.9 mg/l) of purified hydrolyzed *C.I. Reactive Red 120* ($C_{\text{dye}} = 200$ mg/l).

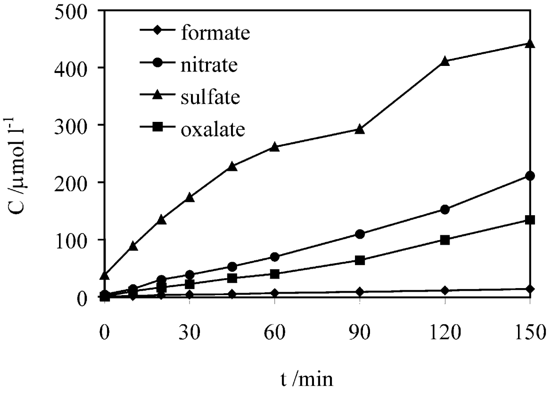

Fig. 4. Concentrations of sulfate, nitrate, formate, oxalate vs the ozonation time (8.9 mg/l) of unpurified hydrolyzed *C.I. Reactive Red 120* ($C_{\text{dye}} = 200$ mg/l).

naphthalene rings lead to increasing amounts of sulfate during ozonation. Apparently, a total of 70.0 and 54.2% of sulfonate were oxidized into sulfate. The amount of sulfate, nitrate, formate and oxalate for ozonation of purified *C.I. Reactive Red 120* is higher than that of unpurified sample, due to different dye contents after purification.

From an ecological point of view, it is necessary to assess the biodegradability of effluents discharged from industrial processes. Results on the investigation of the biodegradability of two different dye samples after ozonation are shown in Figs. 5 and 6 as oxygen consumption during 28 days of incubation. Despite the fact that ozone is a very strong oxidant, complete mineralization of azo reactive dyes in wastewater by ozonation is not achievable [27,28]. Nevertheless, prolonged ozonation may further oxidize some of the newly formed by-products, e.g. oxalate [26,29]. On the other hand, biological treatment of wastewater is the most economic method so that biological removal of the ozonation products following ozonation seems reasonable. Therefore, systematic evaluation of the changes in biodegradability of azo dyes after ozonation is quite necessary as it was attempted in the presented study.

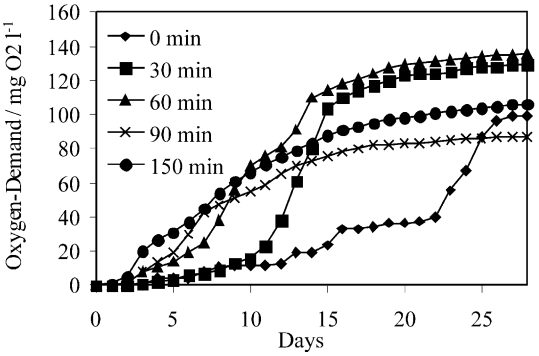

Fig. 5. Oxygen consumption of aqueous solutions of purified hydrolyzed *C.I. Reactive Red 120* during 28 days Sapromat test (C_{dye} = 500 mg/l; ozone concentration = 20.0 mg/l; different ozonation contact times).

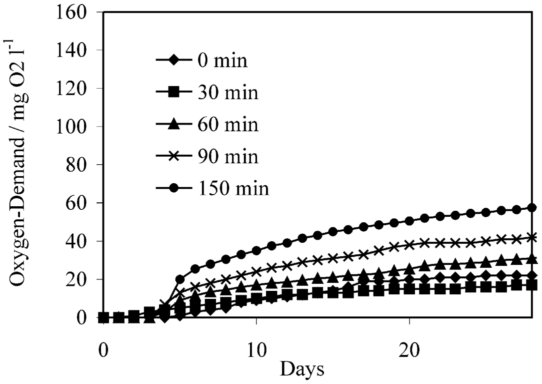

Fig. 6. Oxygen consumption of aqueous solutions of unpurified hydrolyzed *C.I. Reactive Red 120* during 28 days Sapromat test (C_{dye} = 500 mg/l; ozone concentration = 20.0 mg/l; different ozonation contact times).

In general, during biodegradation certain compounds are mineralized and decomposed by transformation since microorganisms use them as carbon and energy sources for growth, converting them to carbon dioxide, new cell material and soluble metabolic products. The problem is, that the majority of the biomass must be capable of using the substrate. This requires the seed culture to be enriched by growth on the substrate of interest prior to its use [30]. In this study, the biomass collected from the effluent of a municipal wastewater treatment plant was not adapted to the studied substrate. As demonstrated in Figs. 5 and 6, an adaptation period of the bacteria to the new environment depending on the ozonation period was necessary. The substrate inhibition can easily be identified from the shape of biodegradation. Results also illustrate that the patterns of biodegradability of the two investigated dye solutions after different ozonation times are different. The oxygen demand of the initial dye solution without ozonation increases after 15 days of incubation, whereas the adaptation period of the bacteria decreases depending on the ozonation time (Fig. 5). It indicates the conversion of the dye molecule to more biodegradable compounds. Samples of unpurified dye solution shows nearly invariable adaptation period of the bacteria during the ozonation (Fig. 6). This could be the effect of easily biodegradable additives/impurities in the commercial dye. The significant increase of mineralization of the initial purified *C.I. Reactive Red 120* (before ozonation) after 22 days incubation (Fig. 5) can be attributed to the adaptation of bacteria to recalcitrant azo dye substrate or so called cross-acclimatization [4].

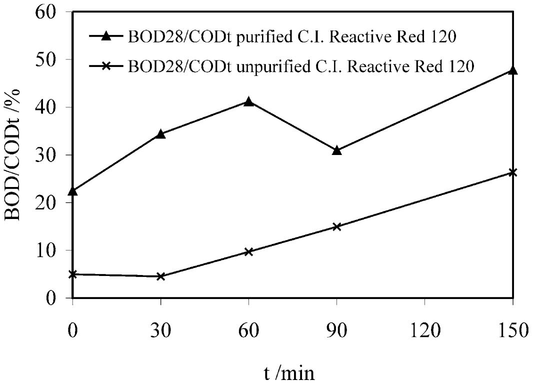

Fig. 7. BOD_{28}/COD ratios during the ozonation of purified and unpurified hydrolyzed *C.I. Reactive Red 120* ($C_{dye} = 500$ mg/l; ozone concentration $= 20.0$ mg/l).

Krull et al. (1998) reported that the biomass used for the anoxic treatment of six different reactive azo dyes was growing in the following aerobic phase when readily biodegradable compounds were present [31]. Using a short-term toxicity test with mixed culture, an inhibition of the respiration activity of 30–85% by the cleft products of e.g. *C. I. Reactive Black 5* at concentrations up to 500 mg/l was observed by the authors. The authors also reported that the mineralization of the remaining recalcitrant organic compounds was improved by partial oxidation with ozone in an aerobic phase. In the present study, the unpurified dye solution after 30 min of ozonation shows slightly less oxygen demand indicating the formation of inhibitory toxic by-products (Fig. 7). On the other hand, as ozonation progresses, the concentration of the toxic intermediates increases inhibiting the biomass growth. Therefore, the biodegradability of the purified *C.I. Reactive Red 120* after 90 min of ozonation is lower than after 30 and 60 min ozonation. After 150 min of ozonation, toxic intermediates are obviously mineralized to smaller organic compounds resulting in a slight increase of biodegradability.

Fig. 7 also illustrates the increase of the BOD_{28}/COD ratio from 0.22 to 0.48 and from 0.05 to 0.26 for purified and unpurified dye sample, respectively. The purified *C.I. Reactive Red 120* shows a higher biodegradability than unpurified *C.I. Reactive Red 120*. The reason could be the existence of dye additives/impurities and their oxidation by-products acting as inhibitors in the case of unpurified dye and affecting the microbial activity. Accordingly, ozonation can be recommended as a partial oxidation step of a combined treatment concept in which ozone can be used as a pretreatment method to reduce refractory substances since ozone is able to transform parts of the organic fraction into biodegradable material.

4. Conclusions

The results of COD, BOD, UV-VIS spectrophotometry measurements and BOD_{28}/COD ratio after 150 min of ozonation of hydrolyzed *C.I. Reactive Red 120*, with and without purification, illustrate the potential of the ozone treatment of dyehouse effluents. Ozonation as an integrative partial oxidative-biological treatment system in order to remove recalcitrant substances from dyehouse effluents can be recommended. For economic and ecologic benefit in a combined system ozonation should convert rather than eliminate recalcitrant substrate for subsequent mineralization by the cheaper biological process.

The results also demonstrate that the dye additives/impurities in commercial dyes affect the microbial activity and as well as the biodegradability of the dye. Final degradation products generated have been identified to be sulfate, nitrate, formate and oxalate as the main oxidation products. It is further concluded that dyes need to be purified before ozonation, if detailed information on the oxidative processes and the resulting oxidation by-products are required.

Further investigations are under consideration to identify the primary oxidation by-products of the commercial azo dyes by HPLC and LC-MS.

Acknowledgements

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