



“Gheorghe Asachi” Technical University of Iasi, Romania



ADVANCED OXIDATION OF AN AZO DYE AND ITS SYNTHESIS INTERMEDIATES IN AQUEOUS SOLUTION: EFFECT OF FENTON TREATMENT ON MINERALIZATION, BIODEGRADABILITY AND TOXICITY

Sanja Papić, Igor Peternel, Željko Krevzelj, Hrvoje Kušić*, Natalija Koprivanac

University of Zagreb, Faculty of Chemical Engineering and Technology, 19 Marulicev trg, 10000 Zagreb, Croatia

Abstract

The objective was to study the degradation of azo dye C.I. Acid Orange 7 (AO7) and intermediates for its synthesis sulphanilic acid (SA) and 2-naphthol (2-N) by Fenton process. Full factorial design and response surface methodology were applied to model the system behavior influenced by studied process parameters ($[\text{Fe}^{2+}]$ and $[\text{H}_2\text{O}_2]$).

Optimal process conditions were determined on the basis of mineralization efficiency. The effectiveness evaluation of applied treatment process was conducted on the basis of UV/VIS, TOC, COD, BOD₅ and toxicity measurements. Fenton reagent ratio 1:62-68 ($[\text{Fe}^{2+}] \approx 1.5$ mM and $[\text{H}_2\text{O}_2] = 100$ mM) yielded the highest TOC reductions for AO7, SA and 2-N (53 %, 44 % and 52 %, respectively), along with the complete decolorization of azo dye solution and degradation of aromatic fragments in solutions after 60-min treatment.

Three synthetic wastewaters are characterized as non-biodegradable before Fenton treatment ($\text{BOD}_5/\text{COD} \leq 0.22$). After 60-min treatment biodegradability of all three wastewaters was improved; BOD_5/COD ratios ranged from 0.38 to 0.52. The values of EC₅₀ showed that all pollutants in investigated concentrations are toxic, while remarkable toxicity reduction after the treatment was noticed only in the case AO7; in the case of other two pollutants the formation of toxic by-products can be assumed.

Key words: azo dye, biodegradability, Fenton process, intermediates, mineralization, toxicity

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1. Introduction

From the start of the synthetic dye industry in 1865 until nowadays several millions of different colored chemical compounds have been synthesized and about 10 000 of these were or are produced on an industrial scale. Azo dyes, characterized by the presence of one or more azo groups (-N=N-) bound to aromatic rings, are the largest and most important class of synthetic dyes. It has been estimated that more than 50% of all dyes in common use are azo dyes because of their chemical stability and versatility. Dyes are widely used in textile, paper, leather, pharmaceutical, cosmetics and food

industries. They are also used in high-tech applications, such as in the medical, electronics, and especially the nonimpact printing industries. However, the scale and growth of the dye industry has been inextricably linked to that of the textile industry. The two most important textile fibers are cotton, the largest, and polyester. Consequently, dye manufacturers tend to concentrate their efforts on producing dyes for these two fibers. The world production of dyes in 1990 was 1×10^6 t. The production of high-tech dyes will remain small in comparison to dyes for traditional applications especially textile dyeing (Hunger, 2002; Neamtu et al., 2002).

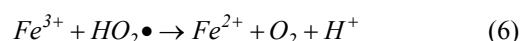
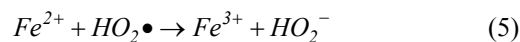
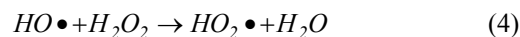
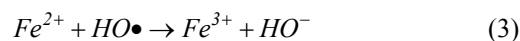
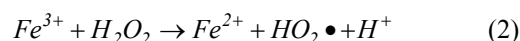
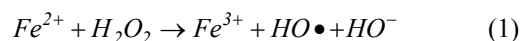
* Author to whom all correspondence should be addressed: e-mail: hkusic@fkit.hr; Phone: +38514597160; Fax: +38514597143

Industrial dyes constitute one of the largest groups of organic compounds that represent an increasing environmental danger. It is estimated that every year worldwide 280 000 t of dyes remain in wastewaters from textile dyeing (Konstantinou and Albanis, 2004; Mass and Chaudhari, 2005; Tantak and Chaudhari, 2006). Most of the dyes used in textile industry are highly stable because they are intentionally designed to resist biological, photolytic and chemical degradation (Arslan-Alaton et al., 2008; Chacon et al., 2006). With regard to environmental, health and safety aspects azo dyes are the most widely investigated class of dyes (Hunger, 2002). Even at very low concentrations (10-50 mg L⁻¹) azo dyes, like other chemical groups of water soluble dyes, can cause waste streams to become highly colored. Aside from their negative aesthetic effects certain azo dyes are considered to be hazardous materials because of their slow biological degradation and of their harmful intermediate degradation products.

Aromatic amines which are formed as metabolites of reductive cleavage of azo bond under anaerobic conditions are more toxic than intact dye molecules (Isik and Sponsa, 2004; Palfi et al., 2011). The direct release of the wastewaters containing water soluble organic synthetic dyes, especially azo dyes, into receiving water body will cause damage to aquatic life. Thus, the treatment of such effluents is important for the protection of natural waters and environment. Due to inefficiency of conventional physical, chemical and biological treatment methods, advanced oxidation processes (AOPs) have become the most promising procedures for purification of dye containing industrial wastewaters and a large number of papers are published on the subject (Arslan and Akmeahmet Balcioglu, 1999; Arslan-Alaton et al., 2009; Hammami et al., 2008; Lucas and Peres, 2006; Nishtar et al., 2008; Wojnarovits et al., 2007).

An important drawback of AOPs is that operational costs are relatively high compared to those of biological treatments, which are the cheapest and the most compatible with the environment. However, the use of AOPs as pretreatment step to enhance the biodegradability of wastewater containing recalcitrant or inhibitory compounds can be justified if the resulting intermediates are easily degradable in a further biological treatment (Garcia-Montano et al., 2006; Sarria et al., 2002). The mechanism of dye destruction in AOPs is based on the formation of a very reactive hydroxyl radicals (HO•), that, with an oxidation potential of 2.80 V, can oxidize a broad range of organic compounds (Andreozzi et al., 1999; Parsons, 2004). Fenton process is a homogeneous catalytic advanced oxidation process using a mixture of hydrogen peroxide (H₂O₂) and ferrous ions (Fe²⁺). Compared with other AOPs, Fenton's reagent is relatively inexpensive and the process is easy to operate and maintain (Neyens and Baeyens, 2003; Zhang et al., 2009). The following reactions (1)-(6) can take place

in Fenton oxidation process (Parsons, 2004; Sun et al., 2009; Sun et al., 2009):



This list includes only reactions taking place between the species in reaction (1) and excludes other species that may be present in the solution. These reactions show that iron acts as a catalyst; however, since the reduction of Fe³⁺ is generally much slower than the oxidation of Fe²⁺ ions, iron exists mainly in the Fe³⁺ form in these systems.

In this study, C.I. Acid Orange 7 azo dye (AO7) and intermediates for its production, sulphanilic acid (SA) and 2-naphthol (2-N) were chosen as model pollutants. Special attention has been paid on the oxidative degradation of AO7, a representative monoazo textile dye, by means of various AOPs including Fenton treatment (Hammami et al., 2008; Ozcan et al., 2009; Ramirez et al., 2009; Styliidi et al., 2004; Velegraki et al., 2006; Zhang et al., 2005; Zhang et al., 2008; Zhang et al., 2009). AO7 can be partially biodegraded by anaerobic treatment to give, by the reduction of azo bond, the primary products sulphanilic acid (SA) and 1-amino-2-naphthol (AN). These products can be very harmful for the ecosystems and human health (Carvalho et al., 2007). Sulphanilic acid, SA, (4-aminobenzensulphonic acid) is a typical representative of aromatic sulphonated amines. Biodegradation of SA is difficult, as the sulpho group is a xenobiotic structural element, and the strongly charged anionic moiety prevents penetration of the compound through bacterial membranes (Perei et al., 2001). Phenols have been listed as priority pollutants by US Environmental Protection Agency and European Union. Naphthalene derivatives with substituents at position 2 are usually more toxic than those at position 1. Due to the strong toxicity and low biodegradability, the removal of 2-naphthol (2-N) is of great importance in water treatment or soil remediation (Zang et al., 2010).

Considering the above mentioned facts, the objective of the present work was to study the degradation of AO7 and intermediates for its synthesis SA and 2-N in aqueous solutions by Fenton advanced oxidation process. Factorial plan (with two factors; concentrations of catalyst Fe²⁺ and oxidant H₂O₂, on three levels of values) in combination with

response surface methodologies (RSM) was used for process optimization relating to TOC removal. The effectiveness evaluation of applied treatment process for all three pollutants was conducted on the basis of UV/VIS, TOC (total organic carbon), COD (chemical oxygen demand), BOD₅ (biochemical oxygen demand) and EC₅₀ (toxicity to the bioluminescent bacteria *Vibrio fischeri*). The conclusions about possibility of combining Fenton process with biological treatment are also presented.

2. Experimental

2.1. Chemicals and solutions

Azo dye Acid Orange 7 (AO7), sulphanilic acid (SA) and 2-naphthol (2-N) were chosen as refractory model pollutants. Dye AO7 was synthesized; SA and 2-N were purchased from Kemika, Croatia. Some important chemical properties of the selected pollutants are presented in Table 1. For the Fenton treatment solutions of AO7, SA and 2-N were prepared in distilled water. The concentration of AO7 was 100 mg L⁻¹ (0.285 mM); concentrations of other two pollutants were also 0.285 mM.

The reason why particularly 100 mg L⁻¹ of AO7 was selected in the present study is that the dye concentration typically encountered in effluents originating from the cotton and polyamide dyeing factories is in the range of 10-200 mg L⁻¹ (Arslan-Alaton et al., 2007). The characteristics of these aqueous solutions prior the treatment are listed in Table 1. The other chemicals used in the present study, also supplied by Kemika, were sodium nitrite (NaNO₂), sodium chloride (NaCl), hydrochloric acid (HCl), sulfuric acid (H₂SO₄), sodium hydroxide (NaOH), ethanol (C₂H₅OH), hydrogen peroxide (H₂O₂; 30% w/w), ferrous iron sulfate heptahydrate (FeSO₄·7H₂O), ammonium metavanadate (NH₄VO₃). All chemicals used were of analytical-grade reagents.

2.2. Synthesis of azo dye C.I. Acid Orange 7

Sulfanilic acid (2 g) was dissolved in 2 M NaOH (5 mL); a solution of NaNO₂ (0.8 g) in water (10 mL) was then added. The resulting mixture was poured in 4 M HCl (10 mL) with stirring and cooling in an ice bath. The temperature of reaction mixture must be around 0-5 °C. After a few minutes suspension of diazonium salt of sulfanilic acid was formed subsequently, this suspension was added with rapid mixing into solution of 2-naphthol (1.6 g) in 2 M HCl (20 mL) at ambient temperature. After a short time crystallization was started and was promoted by adding saturated water solution of NaCl.

Sodium salt of dye was excreted in a form of orange flakes. After filtration, recrystallization was followed: dye was dissolved in a small quantity of hot water, solution was cooled to 80°C and double volume of ethanol was added.

2.3. Reactor and procedure

All Fenton experiments were conducted with 500 mL aqueous solutions of pollutants in a double glass cylindrical jacket reactor, which allows cycle water to maintain the temperature of the reaction mixture constant. A magnetic stirrer was used to stir reaction solutions. Experiments were performed as follows: FeSO₄·7H₂O was added into pollutant solution (AO7, SA or 2-N); pH was adjusted to 3.0 by using the prepared 1.0 M H₂SO₄ or 1.0 M NaOH after which H₂O₂ was added. Treatment process was conducted for 60 min.

In this study two groups of experiments were conducted: (i) first group of experiments in order to find optimal process parameters (Fe²⁺ and H₂O₂ concentrations) for the degradation of each pollutant using the approach based on design of experiments combined with response surface methodology and (ii) second group of experiments in order to establish TOC removal kinetics at found optimal conditions. At the end of each experiment the samples were immediately subjected to TOC measurements. Before characterization of pollutants solutions after Fenton treatment by means of UV/VIS, COD, BOD₅ and EC₅₀ the remaining iron and hydrogen peroxide were removed according to the following procedure. (i) pH was adjusted to 7.0 by using 1.0 M NaOH; the solution was allowed to stand for 24 h in order to remove iron ions by precipitation. (ii) Afterwards, the supernatant was removed, mixed and heated to 60°C for 60 min in order to remove the remaining H₂O₂.

2.4. Analysis

The absorption spectra were recorded by using UV/VIS spectrophotometer Lambda EZ 201, Perkin-Elmer, USA. The mineralization of organic pollutants was monitored on the basis of TOC measurements performed by using a Shimadzu, Japan TOC V_{CPN} 5000 A analyzer. Handylab pH/LF portable pH-meter, Schott Instruments GmbH, Germany was used for pH measurements. The measurements of chemical (COD) and biochemical (BOD₅) oxygen demand were performed by colorimetric method using HACH DR 2800 spectrophotometer, Hach Lange Co, USA, equipped with the barcode reader. For COD and BOD₅ analyses the appropriate Lange reagent kits were used. The toxicity of samples was examined using BioFix Lumi-10 Toxicity Analyzer, Macherey-Nagel, Germany.

The toxicity was evaluated by monitoring changes in the natural emission of the luminescent bacteria *Vibrio fischeri* (freeze-dried, Macherey-Nagel) when treated with toxic compounds. The concentration of H₂O₂ was determined by spectrophotometric method using metavanadate (Pupo Nogueira et al., 2005). The concentration of iron (ferrous and ferric ions) was also determined spectrophotometrically (Clesceri et al., 1998).

2.5. Calculations

In order to investigate the influence of process parameters of Fenton treatment (concentration of Fe²⁺ and H₂O₂) on the degradation efficiency of studied pollutants, as well as to determine the optimal conditions in the investigated range for each of studied pollutants, a 3² full factorial design (FFD) including two numerical factors combined with response surface modeling (RSM) and quadratic programming was applied.

The relationship between the dependent variable(s) and the set of independent variables in RSM is usually given by following quadratic equation (Myer and Montgomery, 2002):

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i=1}^k \sum_{j=1}^k \beta_{ij} X_i X_j + \varepsilon' \tag{7}$$

The terms in Eq. (7) are following: *Y* is the chosen process response (dependent variable); *k* represents the number of patterns; *i* and *j* are index numbers for patterns; β₀ is the offset term, X₁... X_k are coded independent variables; β_{*i*} is the first-order (linear) effect; β_{*ii*} is the second-order (quadratic) effect; while β_{*ij*} represents the interaction effect and ε' is the random error allowing the discrepancies or uncertainties between predicted and observed values. For the purpose of RSM model development, the natural (uncoded) independent variables were transformed in dimensionless coded values at levels according to the chosen experimental design (-1, 0 and 1). The combined influence of ferrous ions (X₁) and H₂O₂ (X₂) concentrations was studied and tested on the mineralization efficiency of studied pollutants after 60 min treatment by Fenton process (*Y*). The full matrix for performed experiments along with the experimental results and observed and predicted values of studied responses for all three pollutants/models are summarized in Table 2.

Table 1. Names, chemical structures, formulas, and properties of studied compounds, as well as characteristics of treated model wastewaters

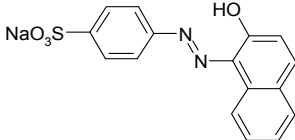
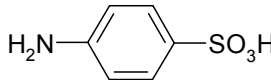
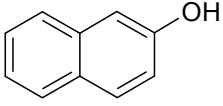
Compound #	1	2	3
Structures, formulas, properties			
Structure			
Name	Orange II (C.I. Acid Orange 7)	Sulfanilic acid	2-Naphthol
Toxicity toward <i>Vibrio fischeri</i> , EC ₅₀ (mg L ⁻¹)	15.83	22.85	1.39
Model wastewaters characteristic			
Molar concentration (mM)	0.285	0.285	0.285
Mass concentration (mg L ⁻¹)	100	49	41
Total organic carbon content, TOC (mg C L ⁻¹)	52.85	20.24	33.81
Chemical oxygen demand, COD (mg O ₂ L ⁻¹)	172.1	64.4	102.6
Biochemical oxygen demand, BOD ₅ (mg O ₂ L ⁻¹)	25.01	4.65	22.7
Toxicity toward <i>Vibrio fischeri</i> , TU (100/EC ₅₀ ,%)	6.32	2.14	29.5

Table 2. 3² Full factorial design matrix with two independent variables expressed in coded and natural units, and values of obtained responses (TOC removal) for three studied pollutants

runs	variables				responses					
	Variable1, X ₁		Variable2, X ₂		Response1, Y ₁		Response2, Y ₂		Response3, Y ₃	
	level	actual value: [H ₂ O ₂], mM	level	actual value: [Fe ²⁺], (mM)	ΔTOC(AO7) ₆₀ , %		ΔTOC(SA) ₆₀ , %		ΔTOC(2-N) ₆₀ , %	
					observed	predicted	observed	predicted	observed	predicted
1	-1	10	-1	0.1	27.40	25.44	13.50	12.97	31.10	29.29
2	-1	10	0	1.05	44.30	46.64	35.50	37.11	47.30	51.86
3	-1	10	1	2.0	41.40	41.02	33.00	31.92	47.50	44.76
4	0	55	-1	0.1	23.50	24.86	15.40	14.78	23.90	23.72
5	0	55	0	1.05	49.60	50.06	40.80	40.94	50.40	48.89
6	0	55	1	2.0	50.26	48.44	37.30	37.78	42.70	44.39
7	1	100	-1	0.1	20.00	20.60	12.50	13.65	22.10	24.09
8	1	100	0	1.05	52.60	49.80	43.60	41.84	54.90	51.86
9	1	100	1	2.0	50.00	52.19	40.10	40.70	48.90	49.96

The fitting of models was calculated using the coefficient of determination R -squared (R^2) and the analysis of variance (ANOVA). For the purpose of ANOVA, regression and graphical analyses of obtained data the software packages STATISTICA 8.0, StatSoft Inc., USA, and Design-Expert 8.0, StatEase, USA, were used. The optimal values of studied process parameters predicted by RSM were calculated using numerical technique built-in software *Mathematica* 6.0, Wolfram Research, USA.

3. Results and discussion

3.1. Optimization of Fenton process by RSM approach

In the first step of the study, the intention was to investigate the combined influence of process parameters of Fenton treatment, Fe^{2+} and H_2O_2 , on the degradation efficiency of all three studied pollutants; azo dye AO7 and two of its intermediates 2-N and SA. In that purpose the statistical/empirical approach including design of experiments combined with response surface methodology was applied. Accordingly, for each of the three studied pollutants 9 independent experiments at set conditions by FFD shown in Table 2 were performed. The results on mineralization extents after 60 min of treatment are correlated with the set conditions using response surface modeling via quadratic polynomial equations calculated for each of the studied pollutants. The obtained polynomial equations, i.e. RSM models M1, M2 and M3 for AO7, SA and 2-N, respectively, are characterized by applying the analysis of variance (ANOVA) to test their significance and fitting. The results are presented in Table 3.

According to the calculated Fisher F -test values along with a low probability values (<0.05), it can be concluded that all three models are significant in predicting the mineralization of all three studied pollutants by Fenton process in dependence of concentrations of ferrous ions and H_2O_2 . The fitting of models to experimental data were tested by

calculating the regression coefficients. It can be seen that in all three cases a rather high regression coefficients ($R^2_{\text{AO7-M1}}=0.979$, $R^2_{\text{SA-M2}}=0.993$ and $R^2_{\text{2-N-M3}}=0.957$) are obtained, indicating the capability of developed models M1, M2 and M3 to satisfactorily describe the system behavior within the investigated range of studied parameters. The same statistical parameters, F and p values, are used to perform rough evaluation of the influence of process parameters on the degradation efficiency. As listed in the Table 3, the concentration of ferrous ions through both linear (X_2) and quadratic (X_2^2) models' terms significantly influences the mineralization of all three pollutants. On the other hand, the influence of H_2O_2 was found significant only in the case of SA (Table 3). These effects can be straightforwardly presented through 3D surface and contour plots (Fig. 1.). It can be clearly observed that the concentration of ferrous ions in all three cases (Figs. 1(A), 1(B) and 1(C) for AO7, SA and 2-N, respectively) significantly influence mineralization efficiency, while influence of H_2O_2 is minor or negligible; the shapes of 3D surfaces are characterized by significantly larger amplitudes in predicted mineralization efficiency when varying the Fe^{2+} concentration than H_2O_2 concentration. Further similarity is related with the optimal Fe^{2+} and H_2O_2 concentrations to achieve the maximal mineralization of all three studied pollutants; on the bases of the contour plots it can be seen concentrations of ferrous ions approximately to 1.5 mM and 100 mM of H_2O_2 would yield highest TOC removal in all three cases. In order to determine the exact optimal studied process conditions in the investigated range for mineralization of all three studied pollutants by Fenton process, the numerical technique built-in *Mathematica* 6.0 software (Wolfram Research, Champaign, IL) was used.

It was found that the highest mineralization extents of AO7, SA and 2-N would be achieved at approximately similar Fenton ratios ($\text{Fe}^{2+}/\text{H}_2\text{O}_2$) ranging from 1:62 to 1:68 and including the 100 mM of H_2O_2 . Calculated values are summarized with the corresponding coded values in Table 4.

Table 3. Analysis of variance (ANOVA) of the response surface models **M1** – **M3** for the prediction of mineralization yield of three studied pollutants after 60 minutes treatment by $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ process

Model	Pollutant	Source	Statistics							Influencing model factors (according to p values)	Influencing process parameters
			SS	df	MSS	F	p	R^2	R^2_{adj}		
M1	AO7	Model	1279.76	5	255.95	27.63	0.0103*	0.979	0.943	X_2, X_2^2	$[\text{Fe}^{2+}]$
		Residuals	27.79	3	9.26						
		Total	1307.55	8							
M2	SA	Model	1278.03	5	255.61	81.10	0.0021*	0.993	0.980	X_1, X_2, X_2^2	$[\text{H}_2\text{O}_2], [\text{Fe}^{2+}]$
		Residuals	9.46	3	3.15						
		Total	1287.49	8							
M3	2-N	Model	1125.36	5	225.07	13.22	0.0295*	0.957	0.884	X_2, X_2^2	$[\text{Fe}^{2+}]$
		Residuals	51.07	3	17.02						
		Total	1176.44	8							

*when $p < 0.05$ model and/or model terms are considered as significant

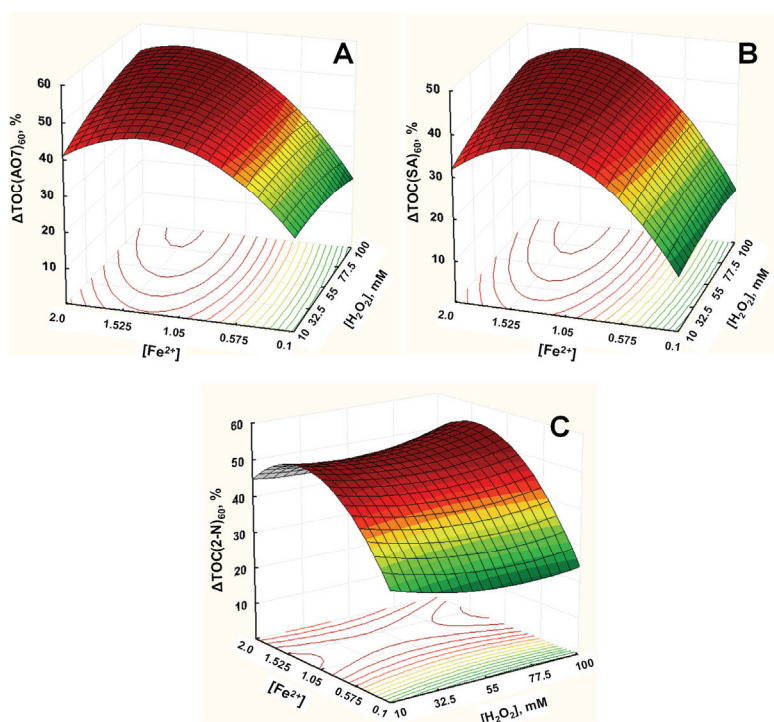


Fig. 1. 3D response surface and contour diagrams showing the effects of the mutual interactions between $[H_2O_2]$ and $[Fe^{2+}]$ on the mineralization yield of three studied pollutants after 60 minutes treatment by Fe^{2+}/H_2O_2 process: (A) AO7, (B) SA, and (C) 2-N

Table 4. Optimal conditions determined by RSM for the each of studied pollutants, as well as predicted and experimentally obtained mineralization yields after 60 minutes treatment by Fe^{2+}/H_2O_2 process at these optimal conditions

pollutant	Optimal conditions			$\Delta TOC_{60\text{min}}$ %	
	$[Fe^{2+}]$, (mM)	$[H_2O_2]$, (mM)	Fe^{2+}/H_2O_2	predicted	observed
AO7	1.61	100	1/62	54.46	53.34
SA	1.49	100	1/67	44.96	44.02
2-N	1.46	100	1/68	54.68	52.75

The RSM models M1, M2 and M3 are verified by the performing the experiments at determined conditions and the results are presented in Table 4, as well. It is evident that the obtained mineralization extents are very close to the corresponding predicted values, confirming validity and high accuracy of developed RSM models for describing treatment of studied pollutants by Fenton processes.

Although the found optimal concentration of H_2O_2 is placed at the boundary conditions in all three cases (100 mM), observing 3D surface plots prediction where H_2O_2 is not marked as highly significant factor, the further increase in its concentration would not yield significant increase of mineralization efficiency, but could yield significant increase in the treatment costs regarding the cost of consumed chemical. Hence, the further experiment in this study where performed at 100 mM of H_2O_2 , and with slightly different concentrations of Fe^{2+} ions found as optimal regarding the type of pollutants. The interesting is that in all three cases similar concentrations of Fe^{2+} ranging from 1.46 to 1.61 mM (and $[H_2O_2]=100$ mM) are found as optimal values (Table 3). Taking into account similar mineralization efficiency in the case of AO7 and 2-N (around 53%)

it can be assumed that in the case of these two pollutants, i.e. parent pollutant and its intermediate, degradation occurred through the similar pathway after initial cleavage of azo bond in the case of AO7. Somewhat smaller degradation extent obtained in the case of SA (44%) support above made assumption, indicating that naphthalene-like by-products of AO7 can be more efficiently mineralized than that benzene-like. In order to elucidate these assumption more deeply, the kinetic of mineralization and degradation of these three pollutants was monitored at determined optimal conditions.

3.2. Degradation and mineralization of AO7 and its intermediates by Fenton process

Optimal conditions (concentrations of Fe^{2+} and H_2O_2) for degradation of AO7, SA and 2-N by Fenton advanced oxidation (Table 4) were found at pH 3. This is because hydrogen peroxide and ferrous ions are more stable when pH is lower than 3.5. As a result, a better redox system and better mineralization efficiency can be established. On the contrary, ferrous ions are unstable when a pH value of the system is higher than 4.0, and they would easily form ferric ions which have a tendency to produce ferric

hydroxy-complexes. The complexes would further form $[\text{Fe}(\text{OH})_4]$ when the pH is higher than 9.0. Besides, hydrogen peroxide is also unstable in basic solution and may decompose to give oxygen and water and lose its oxidation ability (Kuo, 1992). The influence of Fenton oxidation time on the mineralization in terms of TOC removal during the separately treatment of the AO7, SA and 2-N solutions at optimal process conditions established by RSM for each studied pollutant are presented in Fig. 2. After 10 min and 20 min of reaction time the reduction of TOC initial values were about 25 % and 35 %, respectively and were almost equal for all three pollutants. After 60 min TOC removals for AO7, SA and 2-N were 53 %, 44 % and 52 %, respectively. It can also be seen that TOC values measured after removal of iron and hydrogen peroxide (by sedimentation at pH 7 followed by heating to 60°C) after Fenton treatment of AO7, SA and 2-N solutions were not changed. The mineralization is equally for AO7 and 2-N while for SA is 10 % lower. Since the TOC initial value of SA solution is the lowest in comparison with that for AO7 and 2-N solutions, aforesaid small difference could be explained only by more pronounced radical scavenger effect or more strictly, by higher consumption of HO radicals in chemical reactions other than with organic pollutants; most often with Fe^{2+} or H_2O_2 (Eqs. 3 - 4).

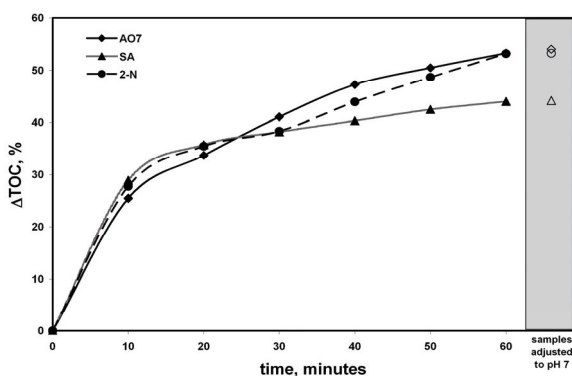


Fig. 2. Mineralization kinetic of three studied pollutants by $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ at determined optimal conditions within the investigated range (AO7 - $\text{pH}_0=3$, $[\text{Fe}^{2+}]=1.61$ mM, $[\text{H}_2\text{O}_2]=100$ mM; SA - $\text{pH}_0=3$, $[\text{Fe}^{2+}]=1.49$ mM, $[\text{H}_2\text{O}_2]=100$ mM; 2-N - $\text{pH}_0=3$, $[\text{Fe}^{2+}]=1.46$ mM, $[\text{H}_2\text{O}_2]=100$ mM)

For the comparison of the TOC removal efficiency in the treatment of AO7 by Fenton or other types of advanced oxidation processes, some examples are found in the literature. The oxidative degradation of AO7 in water was investigated by electro-Fenton technology using electro generated hydroxyl radicals (Hammami et al., 2008; Ozcan et al., 2009). At the optimal experimental conditions ($I = 300$ mA, $[\text{Fe}^{3+}] = 0.1$ mM and pH 3) the almost complete mineralization (92% of TOC removal) of 0.10 mM AO7 aqueous solution was achieved in 8 h by Ozcan et al. (2009). Mineralization of AO7 was

also studied by ultrasound enhanced heterogeneous Fenton-like process (Zhang et al., 2009). Only 42 % TOC was removed even if the reaction time was as long as 90 min when the initial dye concentration was 79.5 mg L^{-1} , H_2O_2 concentration was 7.77 mM, goethite addition was 0.3 g L^{-1} , ultrasonic power density was 80 W L^{-1} , and initial pH value was 3. When the mineralization was tested in combined ultrasound/ozonation process (Zhang et al., 2008) AO7 was nearly mineralized completely after 90 min of reaction when the temperature rose from 29-61°C. The dye concentration was 0.91 mM, initial pH was 4.5, ozone concentration was 0.81 mM and ultrasonic power density was 201 W L^{-1} .

Fig. 3(A) shows typical UV/VIS spectra of aqueous solution of AO7 before treatment and after treatment with and without removal of residual reagents. Due to the interferences of ferric/ferrous ion and H_2O_2 with the aromatic fragments of AO7, SA and 2-N in UV region (200-300 nm), Fe^{2+} and H_2O_2 were removed after 60 min of Fenton treatment (Arslan and Akmehtmet Balcioglu, 1999). The spectrum of AO7 obtained prior to the addition of Fenton reagent ($\text{Fe}^{2+}/\text{H}_2\text{O}_2$) is characterized by one adsorption band in the visible region (485 nm) attributed to the chromophore azo -N=N- group and by two absorption bands in the ultraviolet region at 254 and 300 nm attributed to the aromatic rings (benzene and naphthalene) (Zhang et al., 2008; Zhang et al., 2009).

The dramatic changes in UV/VIS spectra after treatment and removal of $\text{Fe}^{2+}/\text{H}_2\text{O}_2$, represent a disappearance of both azo and aromatic groups. It can be assume that after Fenton treatment of AO7, only ring cleavage compounds like short-chain carboxylic acids are present. The degradation pathway of AO7 mineralization in electro-Fenton treatment is given in the literature (Ozcan et al., 2009). The oxidative degradation of AO7 starts with the breaking of the azo bond, the most active group in the structure, to form sulphanilic acid and 1,2-hydroxy naphthalene. The second stage includes the hydroxylation of these intermediates to hydroxylated or polyhydroxylated derivatives which are oxidized to the quinoid structures. Polyhydroxylated and quinoid structures are unstable and lead to the formation of short-chain carboxylic acids by oxidative ring opening reactions.

Oxidation of the formed carboxylic acids (oxalic, maleic, malonic, formic and acetic acids) to CO_2 constitutes the last stage of mineralization. During the photocatalytic and sonolytic advanced oxidation of AO7 in aqueous solution the following by products are identified: (i) naphthalene-like compounds such as 2-naphthol, (ii) aromatic intermediates such as benzyl alcohol, benzaldehyde, 2-methyl phenol, toluene and benzophenone and (iii) ring-cleavage compounds such as 2-ethyl-1-hexanol and t-butylamine (Velegraiki et al., 2006). Typical UV spectra of SA (Fig. 3(B)) and 2-N (Fig. 3(C)) initial solutions presents absorption bands at 210 and 254 nm and at 254, 280 and 352 nm, respectively.

These bands are attributed to the benzene (254 nm) and naphthalene (325 nm) rings and to the $-\text{NH}_2$, $-\text{SO}_3\text{H}$ (210 nm) and $-\text{OH}$ (280) groups attached to the carbons of the conjugated system. The disappearance of these absorption bands after 60 min of Fenton treatment and $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ removal was considered as evidence of aromatic fragment degradation in SA and 2-N molecules.

3.3. Biodegradability and toxicity of AO7 and its intermediates upon Fenton process

According to the above results, the Fenton process using similar Fenton ratio (1/62-68) shown as efficient for the treatment of wastewater containing AO7, SA and 2-N. The complete degradation of parent pollutants and the majority of their aromatic by-products were achieved according to the disappearance of characteristic absorbance bands for parent pollutants and low A_{254} values in each case (Fig. 3), along with the partial mineralization ($\approx 50\%$). Accordingly, it can be assumed that mostly the aliphatic by-products remained after 60 min treatment.

However, the total mineralization through Fenton process could be hard to achieve and costly through to consumption of additional chemicals due to the complexation of aliphatic by-products with ferric ions forming stable complexes. However, the essential information on the quality of the treated water and the requirements for further treatment or acting with it can be made on the bases of the values of sum-ecological parameters such as: mineralization extent, coloration, aromaticity, biodegradability and toxicity. The efficiency of Fenton process regarding first three ecological parameters is discussed above, while the later two will be examined in the further

text. The most appropriate way to quantify the biodegradability of a studied wastewater is via BOD_5/COD ratio (Sarria et al., 2002). It is commonly accepted that the wastewater with BOD_5/COD higher than 0.4 is biodegradable, while those with ratio between 0.3 and 0.4 are only partially biodegradable (Farré et al., 2007; Sarria et al., 2002).

The changes of BOD_5/COD during the treatment by $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ for model wastewaters containing studied pollutants i.e. AO7, SA, 2-N at determined optimal conditions for each pollutant are shown in Fig. 4. Initial BOD_5/COD ratio values clearly demonstrate that all three pollutants are non-biodegradable before the Fenton treatment. Fig. 4 also shows that the biodegradability of all three pollutants increased after 60 minutes processing by Fenton process.

Furthermore, considering the biodegradability criteria mentioned above model solutions containing AO7 and 2-N model are estimated as biodegradable while the solution containing SA is only partially biodegradable after 60 minutes of Fenton treatment. Accordingly, it can be concluded that further successful treatment can be achieved applying biological methods/processes.

The toxicity of the model wastewaters containing AO7, SA and 2-N prior and after the treatment by Fenton process systems was estimated on the basis of tests performed on a luminescent bacteria *Vibrio fischeri*. The toxicity categories are established by the guidelines (Directive 93/67/EEC) (EEC, 1993), and accordingly AO7 and SA as compounds can be characterized as "harmful to aquatic microorganisms", $\text{EC}_{50}(\text{AO7}) = 15.83 \text{ mgL}^{-1}$ and $\text{EC}_{50}(\text{SA}) = 22.85 \text{ mgL}^{-1}$, while 2-N can be classified as "toxic" one; $\text{EC}_{50}(2\text{-N}) = 1.39 \text{ mgL}^{-1}$ (Table 1).

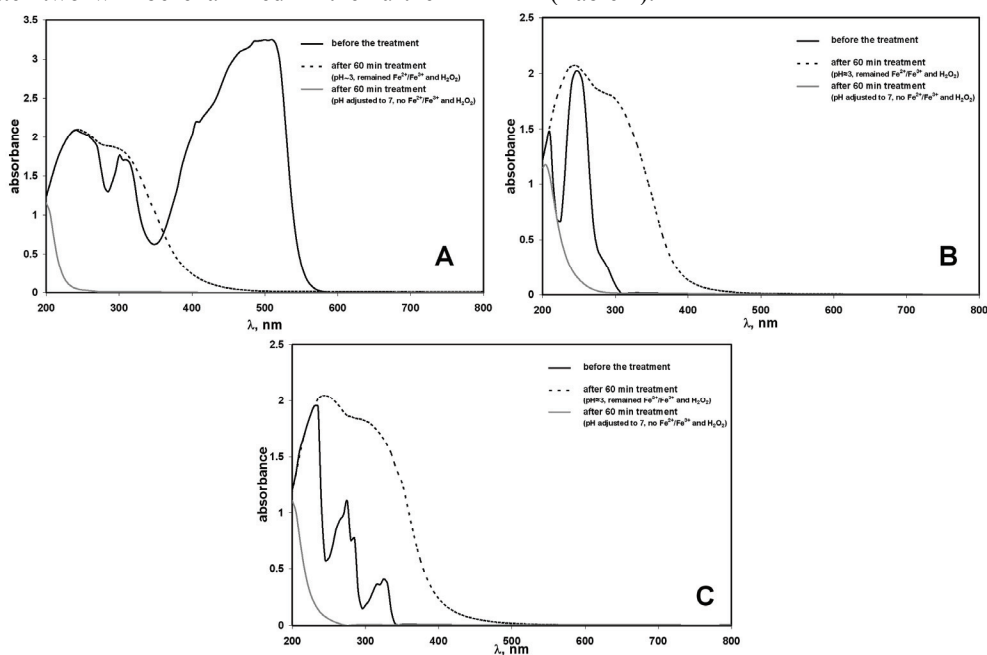


Fig. 3. UV/VIS spectra of the three studied pollutants before and after 60 min treatment by $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ with and without adjustment of pH to neutral and removal of remained iron and H_2O_2 : (A) AO7 ($\text{pH}_0=3$, $[\text{Fe}^{2+}]=1.61 \text{ mM}$, $[\text{H}_2\text{O}_2]=100 \text{ mM}$), (B) SA ($\text{pH}_0=3$, $[\text{Fe}^{2+}]=1.49 \text{ mM}$, $[\text{H}_2\text{O}_2]=100 \text{ mM}$) and (C) 2-N ($\text{pH}_0=3$, $[\text{Fe}^{2+}]=1.46 \text{ mM}$, $[\text{H}_2\text{O}_2]=100 \text{ mM}$)

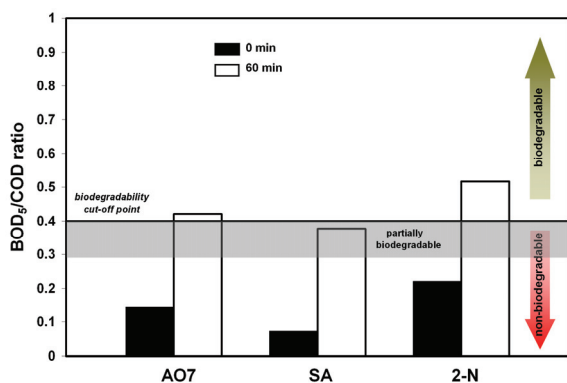


Fig. 4. Changes of biodegradability of model wastewaters containing studied pollutants before (dark) and after 60 minute treatment (white) by $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ process at determined optimal conditions for each pollutant (AO7 - $\text{pH}_0=3$, $[\text{Fe}^{2+}]=1.61$ mM, $[\text{H}_2\text{O}_2]=100$ mM; SA - $\text{pH}_0=3$, $[\text{Fe}^{2+}]=1.49$ mM, $[\text{H}_2\text{O}_2]=100$ mM; 2-N - $\text{pH}_0=3$, $[\text{Fe}^{2+}]=1.46$ mM, $[\text{H}_2\text{O}_2]=100$ mM)

Taking into account the initial concentrations of AO7, SA and 2-N in our model wastewaters, these wastewater samples pertain to the group of the “toxic” (AO7 and SA) and “very toxic” (2-N) ones. The comparison of the toxicity values of these three model wastewaters prior and after the treatment by Fenton process is shown in Fig. 5.

It can be observed that the toxicity decay is higher in the case of AO7 in comparison with 2-N while in the case of SA a strong increase of the toxicity was recorded. The reason for the lower biodegradability as well as for the increase of the toxicity in the case when SA was studied as model pollutant can be due to the formation of amino-compounds that are highly toxic with low biodegradability (Gottlieb et al, 2003; Velegraki et al., 2006). Such compounds can be formed as by-products during the degradation of SA in Fenton treatment (Velegraki et al., 2006; Zhao et al., 2010), thus contributing to lower biodegradability and higher toxicity. It can be assumed that formed amines are presumably of aliphatic nature due to the fact that disappearances of absorbance bands in UV region typical for aromatic compounds was noticed (Fig. 3(B)).

4. Conclusions

In this study Fenton advanced oxidation process was applied for the degradation of azo dye C.I. Acid Orange 7 (AO7) and intermediates for its synthesis sulphanilic acid (SA) and 2-naphthol (2-N) in separate aqueous solutions as model wastewaters. The developed full factorial design and the response surface methodology (RSM) were shown to be appropriate tools for modeling system behavior influenced by studied process parameters. RSM models for AO7, SA, 2-N are significant in predicting the mineralization of studied pollutants by Fenton oxidation in dependence of concentrations of Fe^{2+} and H_2O_2 .

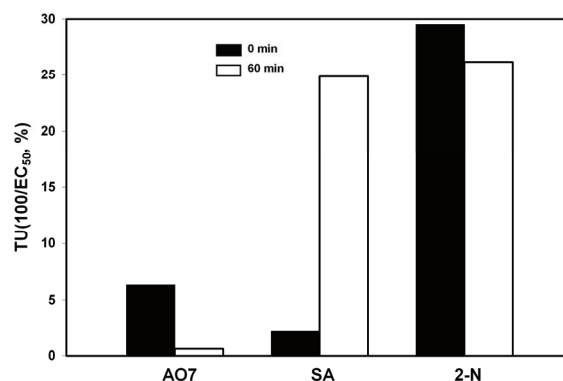


Fig. 5. Changes of toxicity toward *Vibrio fischeri* of model wastewaters containing studied pollutants before (dark) and after 60 minute treatment (white) by $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ process at determined optimal conditions for each pollutant (AO7 - $\text{pH}_0=3$, $[\text{Fe}^{2+}]=1.61$ mM, $[\text{H}_2\text{O}_2]=100$ mM; SA - $\text{pH}_0=3$, $[\text{Fe}^{2+}]=1.49$ mM, $[\text{H}_2\text{O}_2]=100$ mM; 2-N - $\text{pH}_0=3$, $[\text{Fe}^{2+}]=1.46$ mM, $[\text{H}_2\text{O}_2]=100$ mM)

The concentration of Fe^{2+} significantly influences the mineralization of all three pollutants while the influence of H_2O_2 was found significant only in the case of SA. The concentration of Fe^{2+} closely to 1.5 mM (slightly different regarding the type of pollutant) and 100 mM of H_2O_2 yielded the highest TOC reduction in all three cases. After 60 min of treatment at found optimal conditions in the investigated range and $\text{pH}=3$, TOC removals for AO7, SA and 2-N were 53 %, 44 % and 52 %, respectively.

Comparison of UV/VIS spectra of model wastewaters before and after treatment shows a complete color removal which is attributed to cleavage of azo chromophore in dye molecule (AO7) and degradation of aromatic fragments probably to short-chain carboxylic acids (AO7, 2-N, SA).

The biodegradability of studied solutions before and after treatment was quantified by BOD_5/COD ratio. Solutions of all three pollutants were non-biodegradable before Fenton treatment, BOD_5/COD ratios were lower than 0.4. After treatment the solutions containing AO7 and 2-N were estimated as biodegradable (BOD_5/COD ratios were higher than 0.4) while SA solution was only partially degradable (BOD_5/COD ratio was between 0.3 and 0.4). The compounds used in this work as model wastewaters are toxic: $\text{EC}_{50}(\text{AO7})=15.83$ mgL^{-1} , $\text{EC}_{50}(\text{SA})=22.85$ mgL^{-1} and very toxic, $\text{EC}_{50}(\text{2-N})=1.39$ mgL^{-1} . The reduction of toxicity after treatment was higher in the case of AO7 in comparison with 2-N while in the case of SA a strong increase of toxicity was recorded. It is assumed that the reason for the lower biodegradability and higher toxicity of SA solution after oxidative degradation by Fenton process is the formation of some amino aliphatic by products.

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