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Advanced oxidation of acid and reactive dyes: Effect of Fenton treatment on aerobic, anoxic and anaerobic processes

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Abstract

The effect of untreated and Fenton-treated acid dyes (C.I. Acid Red 183 and C.I. Acid Orange 51) and a reactive dye (C.I. Reactive Blue 4) on aerobic, anoxic and anaerobic processes was investigated. The optimum Fe^{2+}:H_2O_2 molar ratio was selected as 1:5 (4 mM:20 mM) for 10 min Fenton treatment at pH 3, resulting in reduced chemical oxygen demand and dissolved organic carbon removal efficiencies; only acetate was detected as a stable dye oxidation end product. During anaerobic digestion, 100, 29% and no inhibition in methane production was observed for the untreated blue, red and orange dyes, respectively. The inhibitory effect of the blue reactive dye on methane production was ~21% after Fenton treatment. Neither untreated nor treated dyes exhibited an inhibitory effect on denitrification. Aerobic glucose degradation was inhibited by 23–29% by untreated dyes, whereas Fenton-treated dyes had no inhibitory effect on aerobic glucose degradation.
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1. Introduction

The textile preparation, dyeing and finishing industry is one of the mightiest water consumers among different industrial sectors and produces 50–100 L wastewater/kg of finished product [1]. From the environmental point of view, particularly the textile dyeing process constitutes a major pollution problem due to the variety and complexity of chemicals (dyes, sequestering agents, tannins, dye carriers, leveling agents, dispersing agents, etc.) employed. Among the textile auxiliaries, dyes have attracted the most attention since color in dyehouse effluent not only causes environmental concerns, but also creates a significant aesthetic problem in sewage treatment works and receiving water bodies [2–4].

Conventional chemical (coagulation–flocculation) and biological (activated sludge, sequential bed reactors, anaerobic/anoxic)-based processes are widely used for textile wastewater treatment, however, with a rather limited success [5,6]. Although some of the more biodegradable dye auxiliaries may be completely eliminated from dyehouse effluent, conventional treatment systems cannot achieve ''destructive'' decolorization due to the fact that textile dyes are intentionally designed to resist biological, photolytic and chemical degradation. The nature of textile effluent depends on fashion, technical, technological, social and economical factors. These effluents often require pre-treatment of segregated process streams (e.g. dyebath effluent) using alternative, advanced oxidation processes (AOPs) that have more recently been used to treat refractory and/or toxic pollutants [7–9].

Among the AOPs, the Fenton's process is quite well known and has been successfully applied for the treatment of dyehouse effluent [10–17]. The Fenton's reagent is relatively cheap and easy to handle compared with other AOPs. Fenton's reagent (being a mixture of H_2O_2 and Fe^{2+} and applied at acidic pH) can be effectively used to achieve complete color and partial COD removal from textile effluent and thus an attractive option to prepare recalcitrant process streams to conventional activated sludge treatment of the combined wastewater. However,

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advanced oxidation products might be more toxic and/or inhibitory on the biological treatment systems used for the post-treatment than the original textile dyes. In fact, many studies have recently reported the effect of Fenton pre-treatment of textile (mainly reactive and acid) dyes on aerobic biological treatment processes by coupling the Fenton (or Photo-Fenton) process with an aerobic biological (mainly sequential batch) reactor; however, no study dealing with the influence of Fenton oxidation of textile dyes on anaerobic and anoxic biological process has been published so far [17–22]. Due to the fact that the textile dyer and finisher is mainly confronted with anaerobic and anoxic treatment systems, it is more interesting and important to study the behavior of these dyes and their degradation products under anaerobic and anoxic conditions.

Considering the above mentioned facts, the aim of the present study was to evaluate the effect of Fenton's treatment of three commercially important textile industry dyes on conventional biological (anaerobic, anoxic and aerobic) processes. For this purpose, two acid dyes, namely Acid Red 183 (AR 183) and Acid Orange 51 (AO 51), and one reactive dye, i.e. Reactive Blue 4 (RB 4), were selected as model textile dyes and subjected to Fenton's treatment under different oxidant (H_2O_2) and transition metal catalyst (Fe^{2+}) concentrations. Fenton treatment efficiency was assessed in terms of color, COD (chemical oxygen demand) and DOC (dissolved organic cabon) removal rates. The study also aimed at quantitatively identifying stable advanced oxidation end products that were expected from textile dye degradation such as carboxylic acids (formic, acetic, malic, maleic, etc.) as well as inorganic salts such as sulfate, nitrite, nitrate and chloride. In the second part of the study, the inhibitory effect of untreated and Fenton-treated textile dyes on biological processes was examined in terms of (a) methane production, (b) denitrification and (c) glucose-COD activated sludge treatment rates.

2. Materials and methods

2.1. Textile dyes

Two acid dyes (AR 183 and AO 51) and one reactive textile dye (RB 4) were chosen as refractory model pollutants and all purchased from Aldrich. Particularly these three textile dyes were selected for this study since their molecular structure is exactly known and all three dyes are frequently being applied for the dyeing of cotton, woolen and nylon (polyamide) fabrics worldwide as well as in Turkey. Some important physicochemical properties of the selected textile dyes are presented in Table 1. For the Fenton treatment as well as anaerobic, anoxic and aerobic experiments, 100 mg/L aqueous dye solutions (corresponding to 171 μM AR 183, 116 μM AO 51 and 157 μM RB 4) were prepared in distilled water. The reason why particularly 100 mg/L was selected in the present study are formerly published papers [17,21,23] as well as private communications with technical staff from local dyehouses [24–26] indicating 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. Environmental characterization of 100 mg/L aqueous solutions of the selected dyes in terms of COD, DOC and absorbance parameters is given in Table 2. The other reagents used in the present study were hydrogen peroxide (H_2O_2; 35% w/w, Fluka), ferrous iron sulfate heptahydrate ($Fe(SO_4)\cdot 7H_2O$, Fluka), and enzyme Catalase (from *Micrococcus lyseidicticus*; 1 AU destroys 1 μmol H_2O_2 at pH = 7 RTP, 100,181 U/mL, Fluka) to destroy residual, unreacted H_2O_2. Peroxide Quant test strips (Aldrich) were used to determine the approximate amount of residual (unreacted) H_2O_2 in the Fenton reaction solutions. Millipore syringe filters with 0.45 μm cutoffs were used to separate supernatant after ferric hydroxide precipitation and to cease the Fenton reaction.

Table 1
Physicochemical properties of the textile dyes AR 183, AO 51 and RB 4

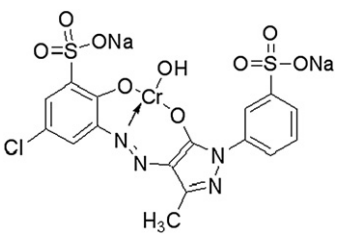
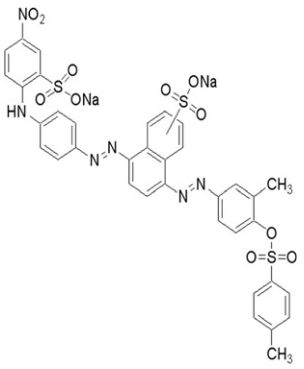
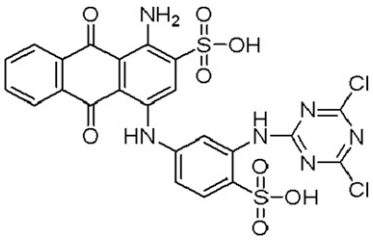
Textile dye	AR 183	AO 51	RB 4
Molecular formula	$C_{16}H_{11}ClN_4Na_2O_8S_2\cdot x$Cr	$C_{36}H_{26}N_6Na_2O_{11}S_3$	$C_{23}H_{14}Cl_2N_6O_8S_2$
Molecular weight (g/mol)	584.84	860.80	637.43
C.I. number	18,800	26,550	2,363,639
λ_{max} (nm)	497	463	595
Molecular structure			

Table 2
Environmental characterization of 100 mg/L textile dye solutions AR 183 (171 μM), AO 51 (116 μM) and RB 4 (157 μM)

Textile dye	AR 183	AO 51	RB 4
COD (mg O_2/L)	50	103	63
DOC (mg C/L)	14	31	21
Absorbance at λ_{max} (cm^{-1})	1.02 at 497 nm	1.75 at 463 nm	0.68 at 595 nm

2.2. Fenton treatment

All Fenton experiments were run with 1000 mL, 100 mg/L aqueous dye solutions at $T = 20\,^{\circ}C$ and an initial pH of 3 in 1500 mL capacity glass beakers. Magnetic stirrers and stir bars were used to provide continuous mixing during the Fenton experiments. In order to initiate the Fenton reaction, appropriate amounts of H_2O_2 stock solution (10.29 M) to achieve the desired H_2O_2 concentration in the final reaction solution, and likewise, $Fe(SO_4)\cdot 7H_2O$ from a 10% w/v (0.36 M) stock solution to achieve the desired Fe^{2+} concentration, were added to the dye solution simultaneously and right after pH adjustment with 0.02, 0.1, 0.5 and 1.0 N H_2SO_4 solutions. Twenty five milliliters of sample aliquots were taken from the beakers at time intervals of 0, 2, 5, 10, 20 and 30 min during the Fenton reaction for analyses. For each sample, the reaction was quenched immediately with the addition of 6 N NaOH solution to increase the pH to around 9–10. Another, second adjustment was done with 0.01 and 0.5 N H_2SO_4 to pH 7–8 in order to achieve the maximum amount of $Fe(OH)_3$ precipitation. After Fe^{2+} was practically completely precipitated out of the reaction solution in the form of $Fe(OH)_3$ (ferric hydroxide) sludge, the sample was filtered through 0.45 μm-cutoff Millipore filters and spiked with sufficient amounts of enzyme Catalase to destroy any residual/unreacted H_2O_2 in order to prevent its positive interference with COD measurements. Catalase also contributed to the COD (1.46 mg COD/mL Catalase) and DOC (1.02 mg DOC/mL Catalase) of the treated samples as determined by preparation of "Catalase control samples" (e.g. H_2O_2 added to distilled water and mixed with exactly the same amount of Catalase that was used to destroy H_2O_2 in the reaction samples). Fenton-treated samples were subjected to color, COD and DOC analyses after filtration.

2.3. Anaerobic experiments

Anaerobic experiments were conducted with untreated dyes as well as dyes being subjected to Fenton's reagent at an initial pH of 3; an Fe^{2+}:H_2O_2 ratio of 1:5 (4 mM:20 mM) and a treatment time of 10 min, for 60 days at $T = 37\,^{\circ}C$ (under mesophilic conditions). The bioreactors were run in triplicate for each untreated and Fenton-treated dye sample. The pH of the dye samples and the anaerobic biomass were adjusted to 7.5 and 6.8, respectively. Before the anaerobic experiments were initiated, oxygen was completely removed from the systems by purging the bioreactors with a mixture of 80% N_2 + 20% CO_2 (v/v). The biomass (determined as 16,200 mg/L in terms of VSS (volatile suspended solids)) used in the anaerobic experiments was obtained from the anaerobic digestor of Lundtofte Urban Wastewater Plant located in Lyngby, Denmark, treating a mixture of primary and secondary sludge. The anaerobic sludge neither was adapted to the dyes nor the Fenton oxidation products both of which consequently served as the sole carbon source. The anaerobic batch reactors schematically presented in Fig. 1(a) consisted of 56, 500–2000 mL capacity glass bottles out of which 10 (total sample volume = 1600 mL) were used for regular color, COD, DOC, VSS and TSS (total suspended solids) analyses and the remaining ones (total sample volume = 300 mL) were kept for regular CH_4 analyses. Formalin (37% w/v, Merck) was added at a concentration of 2% (w/v) to the abiotic control reactors. In addition, control (containing inoculum and distilled water only) and Catalase control (containing inoculum, distilled water, H_2O_2 and enzyme Catalase) reactors were prepared. The "control reactors" refer to the samples that neither bore untreated nor Fenton-treated textile dyes but only water and inoculum. "Catalase control reactors", containing 20 mM H_2O_2 solution spiked with the exact amount of daily prepared, diluted Catalase solution to destroy exactly 20 mM H_2O_2, were also prepared to examine the effect of the Catalase enzyme on anaerobic biomass. The VSS concentration in all bioreactors was adjusted to 500 mg/L. No additional external carbon source was used in the bioreactors to observe the effect of the untreated and treated dyes on the methanogenic activity more clearly. However, no methane production was observed within the first 48 h of the anaerobic experiments in all batch reactors including controls. For this reason, 500 mg/L glucose solution was added to one of the control bioreactors to observe if the delay in methane formation was related to the absence of readily degradable substrate. Samples were taken at regular time intervals up to 60 days for color, COD, DOC, VSS and TSS analyses.

2.4. Anoxic experiments

Anoxic experiments were conducted with untreated dyes as well as dyes being subjected to Fenton's treatment ($pH_o = 3$; Fe^{2+}:H_2O_2 = 4 mM:20 mM; $t = 10$ min) for 48 h at $T = 20\,^{\circ}C$. For the anoxic experiments, sludge (VSS = 6400 mg/L) was collected from the anoxic/aerated tank after the primary settling tank of Lundtofte Urban Wastewater Plant ("Biodenitro" plant) located in Lyngby, Denmark. The pH of the untreated/treated dye samples and the biosludge were initially adjusted to 7.5 and 6.8, respectively. Sludge was not adapted to the dyes and Fenton oxidation products. At the beginning of the denitrification experiments, $NaNO_3$ (Fluka) and $NaCH_3COO$ (Riedel) were added to the reactors at concentrations of 111.8 mg/L (1.31 mM) and 335.6 mg/L (5.69 mM), respectively. The VSS concentration in all bioreactors was selected as 2400 mg/L. The anoxic batch reactors (one typical reactor is depicted schematically in Fig. 1(b)) consisted of 14, 2000 mL capacity glass bottles (total sample volume = 1600 mL) from which sample aliquots were taken every half an hour during the first 4 h of the experiment and then after 24 and 48 h for color and nitrate analyses. Pure argon (100% v/v) was used to remove nitrogen, oxygen and other

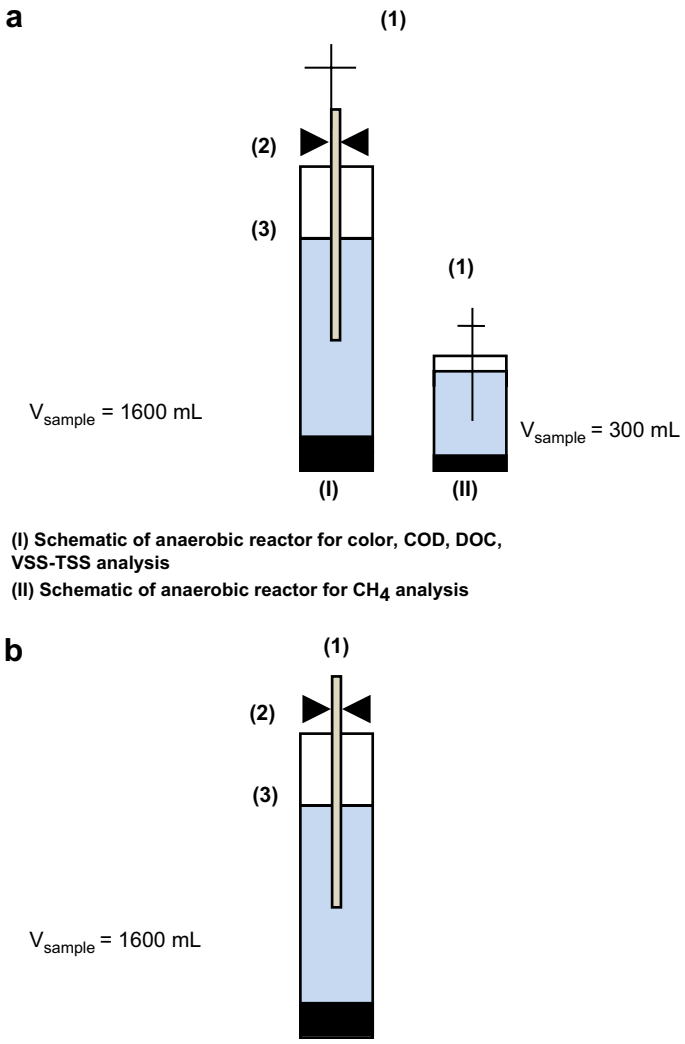


Fig. 1. Schematic representation of the (a) anaerobic and (b) anoxic experimental set-ups. Elements of the anaerobic and anoxic reactors: (1) Sampling syringe; (2) valve to maintain a closed system; (3) tubing providing the connection between sample and syringe.

gases in the bioreactors before the experiments were started. Formalin (37% w/v) was added to abiotic reactors at a concentration of 2% v/v. In addition, separate control and Catalase control reactors were run in parallel as described for the anaerobic experiments.

2.5. Aerobic experiments

Aerobic experiments were conducted separately for untreated dyes and Fenton-treated oxidation intermediates to examine their effect on glucose degradation with heterotrophic biomass. For that purpose, activated sludge experiments were run for 6 h in 8, 1000 mL fed-batch reactors at $T = 20$ °C and pH = 7.0–7.5 with mixed bioculture that was obtained from the aeration tanks of Tuzla and Baltalimanı Urban Wastewater Treatment Plants located in Istanbul, Turkey. The activated sludge was first acclimated to glucose solutions (COD_o (initial COD) = 500 mg/L) for 8 weeks and thereafter used in the aerobic experiments. Samples containing 516 mg/L glucose (=500 mg/L COD) and 100 mg/L untreated textile dyes or textile dyes being subjected to Fenton treatment ($pH_o = 3$; Fe^{2+}:H_2O_2 = 4 mM:20 mM; $t = 10$ min) were individually prepared together with their corresponding controls (516 mg/L glucose only (=500 mg/L COD), appropriate pH buffers and macro/micronutrients. The VSS concentration was kept at 4400 mg/L for the experiments with untreated and 3700 mg/L for the experiments with Fenton-treated dyes to maintain the F/M (food-to-microorganism) ratio in the aerobic sample and control bioreactors at 0.12 and 0.13 mg COD_o/mg VSS, respectively. During the aerobic experiments, sample aliquots were taken at periodic time intervals up to 6 h for color (absorbance), COD, TSS and VSS analyses after filtration through 1.20 (Whatmann) and 0.45 (Millipore) μm-cutoff filters. Again, control and Catalase control reactors were prepared as described for the anaerobic as well as anoxic experiments.

2.6. Analytical tools

In this study, color was referred to as the absorbance measured at the maximum absorption bands of the investigated

textile dyes (i.e. 497 nm for AR 183, 463 nm for AO 51 and 595 nm for RB 4). The absorbance of the untreated and treated samples was measured on a Jenway 6400 model UV–vis (ultraviolet–visible) spectrophotometer at the Technical University of Denmark (DTU), Denmark, and on a Novespec II/Pharmacia LKB colorimeter at Istanbul Technical University (ITU), Turkey. Absorbance measurements were performed with 1 cm optical path length, reusable glass cells. COD measurements were based on Danish Standard (DS) No. 217 [27] for low-range (15–100 mg O_2/L) COD at DTU, Denmark, whereas COD measurements were conducted in accordance with ISO 6060 [28] by the closed reflux, titrimetric method at ITU, Turkey. DOC was measured on a Shimadzu TC (total Carbon) 5000A carbon analyzer after filtration of the samples through 0.45 μm Millipore syringe filters. In order to calibrate the instrument, several dilutions of potassium hydrogen phthalate and potassium bicarbonate solutions were prepared using certified references for TC and IC (Inorganic Carbon) analyses (500 mg/L each, QC WW4A Eurofins). An ICS-1500 model Dionex ion chromatograph equipped with an Ion Pac AS 14 mm (10–31) Column (P/N 46124) in combination with an anion suppressor (AMMS III 4-mm P/N 56750) was used to measure nitrite, nitrate and sulfate and carboxylic acids in the untreated and treated samples during Fenton and anoxic experiments. Column and cell heater temperatures were set as 35 °C, whereas the column pressure was selected as 1400 psi. A Shimadzu model-14A gas chromatograph equipped with a Porapak 60/80 Molsiere column (6 ft in length and 3 mm in inner diameter) and a flame ionization detector (FID) was used for CH_4 measurements during the anaerobic experiments. Nitrogen (100% v/v) was used as the carrier gas at a pressure of 2.0 kg/cm^2. The injection temperature was set as $T = 110$ °C, while the detector and oven temperatures were adjusted to 160 °C. The retention time for CH_4 was around 50 s. A GC Shimadzu GC-2010 equipped with a capillary column (ZB-FFAP, 30 m × 0.53 mm × 1.0 μm) and an FID were used to measure acetate during anaerobic and Fenton experiments. TSS and VSS were measured according to a procedure outlined in Standard Methods [29]. pH was measured using digital pH meters (Metrohm, model 692, at DTU and Thermo Orion, model 520 at ITU). The chromium content of AR 183 before and during Fenton treatment was analyzed on a Unicam 929 model atomic absorption spectrometer with and without 50% v/v nitric acid digestion to determine the total amount of chromium and chromium released from the organically bound trivalent chromium complex azo dye during Fenton oxidation, respectively. Unfortunately, chromium leached out of the chromium complex acid dye directly after acidification with 14.5 N HNO_3, even before the dye was subjected to Fenton's treatment. The total chromium content was determined as 4.5 mg/L in the 100 mg/L digested, original dye sample and 3.8 mg/L in the undigested dye sample. Chromium levels decreased to 1.2 mg/L for Fenton-treated dyes that might be attributable to adsorption of the released chromium ions and/or organically bound chromium on $Fe(OH)_3$ sludge that was formed during precipitation of $Fe^{2+/3+}$ ions to cease the Fenton reaction and to remove residual soluble iron.

3. Results and discussion

3.1. Fenton experiments: establishment of optimum working conditions

The main process variables affecting the rate of Fenton's reaction are the molar concentrations of the oxidant (H_2O_2) and catalyst (Fe^{2+}), particularly the Fe^{2+}:H_2O_2 molar ratio. Increasing the H_2O_2 concentration is important to obtain high oxidation efficiencies, while elevating the Fe^{2+} concentration directly enhances the oxidation rate [11,13,16,30]. However, it should be kept in mind that if the concentration of one reactant is increased to observe its positive effect, thereby keeping the other one constant, the Fe^{2+}:H_2O_2 molar ratio and hence the oxidation condition will change significantly. When one of the reagents is provided in excess, a dramatic reduction in the oxidation efficiency is expected. This can be explained by the following scavenging reactions of $^{\bullet}OH$ (hydroxyl radicals) [31]:

$$Fe^{2+} + {}^{\bullet}OH \rightarrow Fe^{3+} + OH^- \quad (k_{20^\circ C} = 4.3 \times 10^8\ M^{-1}\ s^{-1}) \tag{1}$$

$$H_2O_2 + {}^{\bullet}OH \rightarrow H_2O + HO_2^{\bullet} \quad (k_{20^\circ C} = 2.7 \times 10^7\ M^{-1}\ s^{-1}) \tag{2}$$

Generally speaking, advanced oxidation of organic compounds is fast when ferrous ion is present at a concentration varying between 2 and 5 mM, e.g. a concentration range where sufficient $^{\bullet}OH$ are produced and Fe^{2+} is still highly soluble in water at pH 2–5. In the present study, a set of preliminary (baseline) Fenton experiments was carried out with 100 mg/L aqueous AR 183, AO 51 and RB 4 solutions at an initial pH of 3 (i.e. the optimum pH for Fenton reactions) at different Fe^{2+}:H_2O_2 molar ratios selected as 2:20, 2:40, 4:20 and 4:40 (in mM). These selected Fe^{2+} and H_2O_2 concentrations are corresponding to optimum Fenton molar ratios (1:5 and 1:10). The most suitable molar ratio for each dye was individually determined upon inspection of the obtained color and COD–DOC removal rates. In order to examine the extent of degradation, possible nitrite, nitrate, sulfate and carboxylic acid formation was also followed. Table 3 summarizes percent COD and DOC removal efficiencies obtained after 10 and 30 min Fenton treatment for all the three studied textile dyes. Since color removal was very fast and practically complete (>95%) within the first 2 min of the reaction, color abatement was not further considered as a critical process parameter in these baseline experiments. From Table 3 it is evident that treatment efficiencies generally speaking increased with increasing Fe^{2+} and H_2O_2 concentrations, as expected. However, the improvement in treatment efficiencies was more dramatic when either the catalyst or the oxidant concentration was doubled at the lower concentration of the other reagent (2 mM in the case of Fe^{2+} and 20 mM for H_2O_2). This was particularly evident for the Cr(III) complex azo dye AR 183 and the anthraquinone dye RB 4. AO 51 was not so seriously affected by the variations in molar

Table 3
Results obtained for the baseline Fenton experiments conducted with 100 mg/L aqueous AR 183 (171 μM), AO 51 (116 μM) and RB 4 (157 μM) at different Fe^{2+}:H_2O_2 molar ratios and an initial pH of 3

Fe^{2+}:H_2O_2 (mM:mM)	COD removal[a] (%)		DOC removal[a] (%)	
	t_{10}	t_{30}	t_{10}	t_{30}
AR 183				
2:20	32	38	28	32
2:40	50	56	42	49
4:20	56	58	50	54
4:40	55	57	47	50
AO 51				
2:20	66	72	57	67
2:40	67	78	64	66
4:20	75	80	75	76
4:40	70	77	70	74
RB 4				
2:20	38	38	28	32
2:40	48	56	30	49
4:20	66	58	47	53
4:40	57	57	40	43

[a] Percent COD and DOC removal after 10 min (t_{10}) and 30 min (t_{30}) Fenton treatment at $pH_o = 3$.

concentrations and ratios of Fe^{2+} and H_2O_2 implying that even the lowest tried doses were sufficient for partial oxidation of this disazo dye. However, COD and DOC removal efficiencies even started to decrease when the Fe^{2+}:H_2O_2 molar ratio was increased from 1:5 to 1:10 for 4 mM Fe^{2+}, indicating that H_2O_2 was ''overdosed'' when its concentration was increased to 40 mM. From these findings it can also be inferred that the most suitable Fe^{2+}:H_2O_2 molar ratio was found to be 1:5 at Fe^{2+} and H_2O_2 concentrations of 4 and 20 mM, respectively, coinciding with the highest COD and DOC removal efficiencies for both 10 and 30 min Fenton treatment. In a related investigation conducted with the Fenton's reagent, an optimum molar ratio of 1:4 was established for the treatment of simulated acid dyebath effluent bearing azo and anthraquinone dyes [16]. In another study, the optimum molar ratios were determined as 1:5 and 1:7 for the Fenton oxidation of Disperse Blue 106 and Disperse Yellow 54, respectively [13]. The differences in treatment efficiencies may be attributed to the differences in their molecular structures and molar concentrations. AO 51 has two azo bonds compared to the single azo bond of the chromium complex azo dye AR 183 and an appreciably higher molecular weight than AR 183. The molar concentration of AO 51 is conclusively less than that of AR 183.

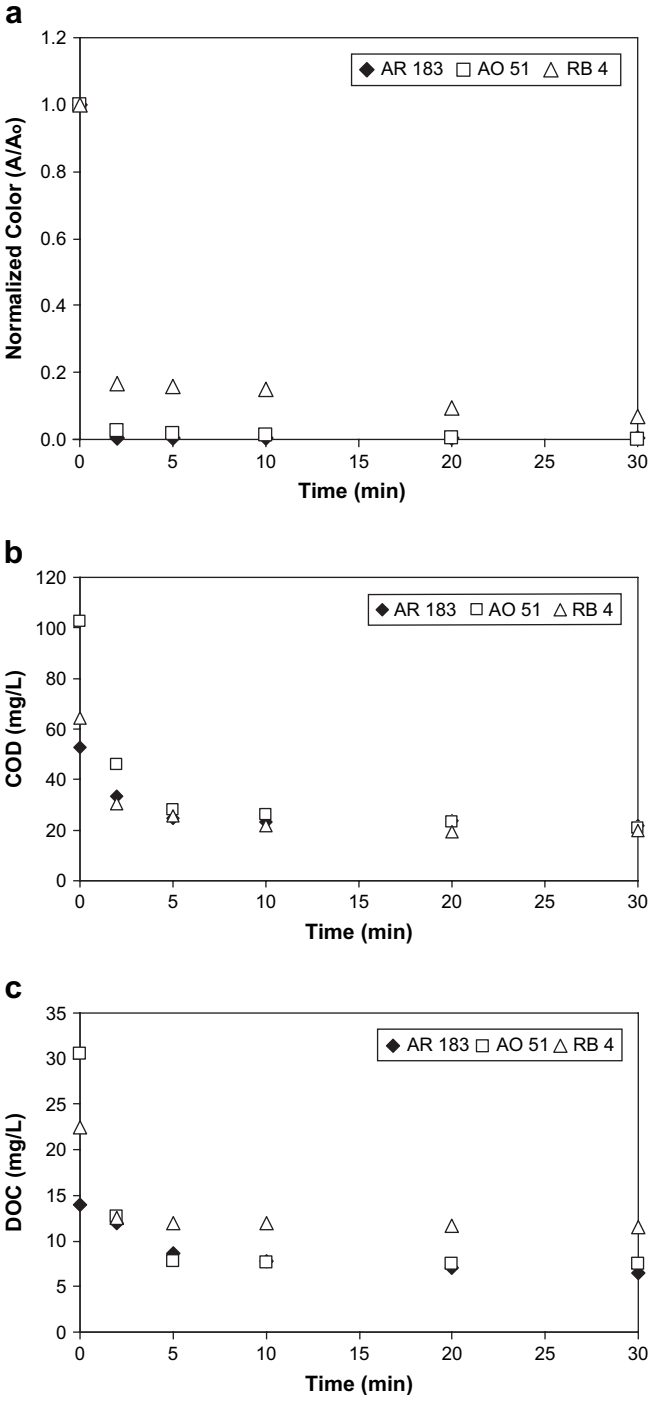


Fig. 2. (a) Normalized color, (b) COD and (c) DOC abatement during Fenton treatment of 100 mg/L AR 183, AO 51 and RB 5 ($Fe^{2+} = 4$ mM; $H_2O_2 = 20$ mM; $pH_o = 3$).

3.2. Fenton treatment

3.2.1. Color

Considering the results obtained in the preliminary Fenton experiments, it was decided to continue the study under the following reaction conditions; initial pH (pH_o) = 3; $Fe^{2+} = 4$ mM and $H_2O_2 = 20$ mM. Fig. 2(a) presents color abatement rates for the three textile dyes under the above mentioned working conditions. From Fig. 2(a) it is obvious that color removal was practically complete within the first 2 min of Fenton reaction. For the azo dyes (AR 183 and AO 51) 99% and for the reactive dye RB 4 93% color removal was achieved after 30 min, respectively. The rapid decolorization rates can be explained by the fast reaction between Fe^{2+} and H_2O_2 to produce a sufficient amount of $^{\bullet}OH$ that promptly cleaved the dye chromophores. In other words, the in situ formed oxidants (free radicals such as $^{\bullet}OH$ and $HO_2^{\bullet}$) had

enough oxidation capability to degrade the chromophores of all of the studied dyes at a high degree (>90%). From previous studies reported in the literature, fast and complete color removal (>99.9%) was achieved by employing Fenton's reagent for 50 mg/L Acid Orange 7 [21]. Similarly, color removal was obtained as 96% for 50 mg/L Reactive Black 5 during the first minute of Fenton's reaction [17]. As is also evident from Fig. 2(a), color removal proceeded so fast that is was not possible to fit the color abatement curve to any reaction kinetic.

Fast color removal was accompanied with a parallel, rapid decrease in H_2O_2 concentration (only 40% of the initial H_2O_2 remained in the reaction solution after 5 min Fenton treatment and was almost completely consumed at the end of the treatment process) indicating rapid consumption of the Fenton reagent as the oxidation proceeded. Indeed, fast consumption of H_2O_2 during the early stages of textile dye degradation via Fenton treatment has been reported before by many researchers [21,32,33].

3.2.2. COD

Fig. 2(b) displays changes in COD during Fenton treatment of the studied textile dyes. From Fig. 2(b) it is evident that COD abatement started right after initiation of the reaction and slowed down after only 5 min treatment most probably due to the accumulation of advanced oxidation intermediates that are more resistant to further oxidation than the textile dyes, as well as completion of the decolorization process. In all cases, only partial COD removal was achieved at the end of the reaction due to the fact that after cleavage of the dye chromophores the reaction slows down such that the highly complex-structured dye molecules are only partially degraded to relatively small organic fragments, such as carboxylic acids, aldehydes, ketones and alcohols [34,35]. For this reason, complete oxidation (mineralization) of the dyes was not expected. This observation can be supported by a former, related work where Fenton oxidation of synthetic acid dyebath effluent resulted in only 23% COD removal after 30 min treatment [16]. In another investigation which was carried out with real, combined textile wastewater, only 30% COD removal could be achieved after Fenton treatment. This behavior was attributed to the relatively short retention time selected for the treatment process and low reagent doses leading to incomplete degradation and accumulation of stable oxidation intermediates in the reaction medium [12].

3.2.3. DOC

DOC abatement of the three textile dyes was also followed under the Fenton treatment conditions and shown in Fig. 2(c). From Fig. 2(c) it is apparent that results obtained for DOC and COD removals were almost parallel to each other; DOC abatement proceeded very fast and showed an asymptotic behavior during the first 5 min of the reaction, speculatively due to accumulation of oxidation intermediates and consumption of the Fenton reagents. As valid for color and COD removal rates, the decreasing order of DOC abatement rates was obtained as AO > AR > RB. This observation is a consequence of differences in molecular weights and structures; it is known that the azo bond is more prone to oxidative attack by $^{\bullet}OH$ than the anthraquinone-based chromophoric grouping [5].

3.2.4. Identification of final (stable) oxidation products

Mechanistic studies were mainly devoted to the ozonation of azo dyes and their number increased recently mainly because case studies dealing with the effect of advanced oxidation processes on the toxicity of textile dyes demonstrated that toxicity may increase if treatment conditions are not optimized and/or treatment time is kept too short. Analytical studies conducted to elucidate the degradation mechanism of textile dyes have been rather qualitative than quantitative, based on the assumption that $^{\bullet}OH$ play a major role in the degradation pathway. Former studies have indicated that $^{\bullet}OH$-addition to N-positions (in the azo bond) and different C-positions (in the phenol and naphthol rings), that, with the help of the hydrogen radical, were subsequently cleaved to intermediates such as 2-hydroxynaphthaquinone, hydroquinone, benzo-1,4-quinone, naphthalene-2-sulphonic acid, urea, 1-naphthol and acetamide, is the initial step of oxidative dye degradation. The benzene rings were further cleaved to yield phthalic acid and/or but-2-enedioic acid and finally degraded to aliphatic carboxylic acids such acetic and formic acid. Most intermediates that are probably involved in the degradation of azo dyes remain tentative states and could not even be qualitatively determined via ion chromatography (IC) and gas chromatography/mass spectrometry (GC/MS) [36]. In another study it was anticipated that during advanced oxidation of metal-complex azo dyes the rupture of azo and metal oxygen groups takes place initially, followed by further oxidation of the aromatic compounds to alcohols, aldehydes and carboxylic acids. Released metal ions (including cobalt, copper and chromium) could be effectively removed via metal hydroxide precipitation under alkaline conditions. In a recent investigation, GC/MS analysis of the cobalt-complex azo dye Acid Brown 159 that was subjected to ozonation and Fenton treatment, resulted in the identification of acetophenone, 1-methyl-1-benzimidazol-2-amine, *N*-(3,4-dimethyl-2,6-dinitrobenzyl) pentan-3-amine and *N*-methyloaniline [37] as the dye intermediates. On the other hand, initial ozonation products of Reactive Red 120 were identified as phenol, 1,2-dihydroxysulfobenzene and 1-hydroxysulfonbenzene via high performance liquid chromatography (HPLC)/mass spectrometry (MS/MS) [38]. Final and hence smaller molecular-weight oxidation products were detected as acetate, sulfate and nitrate in that study as many reactive dyes, Reactive Red 120 comprises azo, triazine and amino groups. However, in these studies it is believed that the nitrogen in the azo bond is converted to nitrogen gas (molecular nitrogen) since nitrate could not be detected or only in trace amounts during advanced oxidation and ozonation of many azo dyes including Reactive Red 120 and Congo Red [39,40]. Harsh oxidation conditions were needed for the oxidation of compounds bearing 1,3,5-triazinyl-groups such as (amino)chlorotriazine reactive dyes [38,41]. In another recent investigation, solid-phase extraction coupled with GC/MS analyses was employed to identify dye intermediates during advanced oxidation of Reactive Orange 113. Octanal,

acetophenone, nonaldehyde, butylethyl acetic amide, 1,2-benzenedicarboxylic acid, diethylester, as well as substituted phenols and amides were detected and a possible degradation pathway was postulated. In that experimental study, it was demonstrated that naphthalenes were degraded to substituted benzenes and alcohols, and substituted benzenes on the other hand were cleaved to aldehydes and carboxylic acids. Again, the triazine group remained intact during ozonation and the Fenton process. Neither ozonation nor Fenton process was capable of achieving complete mineralization (TOC abatement was always less than 40%) [42]. The present investigation aimed at quantifying stable oxidation intermediates (low-molecular-weight carboxylic acids such as formic, malic, maleic, acetic acid) and oxidation end products (such as sulfate, nitrite, nitrate, chloride, etc.). However, none of these compounds with the exception of acetic acid and sulfate was quantifiable at significant levels. Sulfate results were rather meaningless since ferrous sulfate was employed as the ferrous iron source for the Fenton experiments and remained constant throughout the Fenton process, whereas the concentrations of nitrite and nitrate were always found less than 0.2 mg/L. No low-molecular-weight carboxylic acid other than acetic acid could be detected during Fenton treatment of the three textile dyes. Hence, Fig. 3 only depicts acetate formation (a) and its contribution to the total DOC (b) remaining from the degradation of the three textile dyes via Fenton oxidation under predetermined reaction conditions. In all cases, acetate concentrations were less after 30 min as compared with the acetate concentration found after 10 min Fenton treatment. This

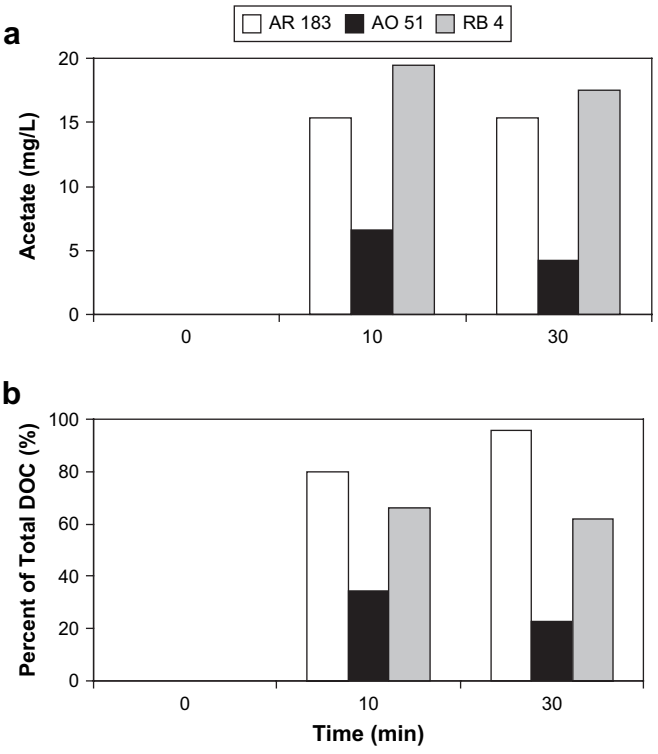


Fig. 3. (a) Acetate formation and (b) acetate-DOC given as a fraction of the total DOC during Fenton treatment of 100 mg/L AR 183, AO 51 and RB 5 ($Fe^{2+} = 4$ mM; $H_2O_2 = 20$ mM; $pH_o = 3$).

observation may be attributable to its gradual conversion to CO_2 and H_2O as the reaction proceeded. The decreasing order of acetate formation rate was observed to be inversely proportional to the decreasing DOC abatement rates, namely RB 4 > AR 183 > AO 51. This may be explained as follows: Fenton oxidation was appreciably faster for AO 51 than for the other two dyes and hence a significant portion of the formed acetate (advanced oxidation intermediate) was already converted to CO_2 (oxidation end product) and H_2O upon extension of the reaction time from 10 to 30 min. Ninety six percent of the final DOC originated from the acetate that was formed during Fenton oxidation of AR 183. Almost 20 mg/L acetate accumulated in the reaction solution when RB 4 dye was subjected to Fenton oxidation for 10 min and decreased to 17 mg/L after 30 min treatment time.

3.3. Anaerobic experiments

3.3.1. Color

Changes in color during anaerobic experiments of untreated AR 183, AO 51 and RB 4 are given in Fig. 4(a). Initial sorption onto biomass (the absorbance removed before the experiment was initiated via physical adsorption) accounted for 15, 65 and 25% AR 183, AO 51 and RB 4 removals, respectively (not shown data), while by total biosorption (preliminary adsorption plus biosorption during the experiment) 46% (AR 183), 89% (AO 51) and 66% (RB 4) color removals were achieved after 60 days of anaerobic digestion. It is known that the decolorization of azo dyes under anaerobic conditions may involve both biosorption and reductive cleavage of azo bonds. Biosorption is a complex function of the biomass type, structure, surface properties, surface area and concentration, exposure time to the dyes as well as dye concentration, molecular structure, polarity, and other physicochemical properties. Some researchers established that in the presence of an appropriate carbon source, anaerobic color removal is mainly due to azo dye reduction [43,44]. In a study conducted with Direct Black 38 at a concentration range of 100–3200 mg/L and 2000 mg/L glucose-COD added as the co-substrate, color removal was found 80–100% after 15 h [45], whereas Albuquerque et al. [46] observed 90% decolorization of 25 mg/L Acid Orange 7 in the presence of 1150 mg/L starch (1000 mg/L COD) that served as the external carbon source after only 10 h. On the other hand, Maas and Chaudhari [47] who examined the anaerobic decolorization of 100 and 200 mg/L Reactive Red 2 found that biosorption played a major role in color abatement and accounted for more than 80% removal of the original dye. The overall decolorization efficiency was obtained as 78 and 76% after 27 days for 100 and 200 mg/L Reactive Red 2, respectively. In another work carried out with Reactive Blue 5 at a concentration of 100 mg/L, color removal was 37% after 16 days [48] and it could be demonstrated that decolorization was mainly due to biosorption. The absence of an external co-substrate in the present study could have resulted in an inefficient decolorization process via reductive cleavage. This can be supported by a study which was conducted with Disperse Blue 79; for low concentrations of this

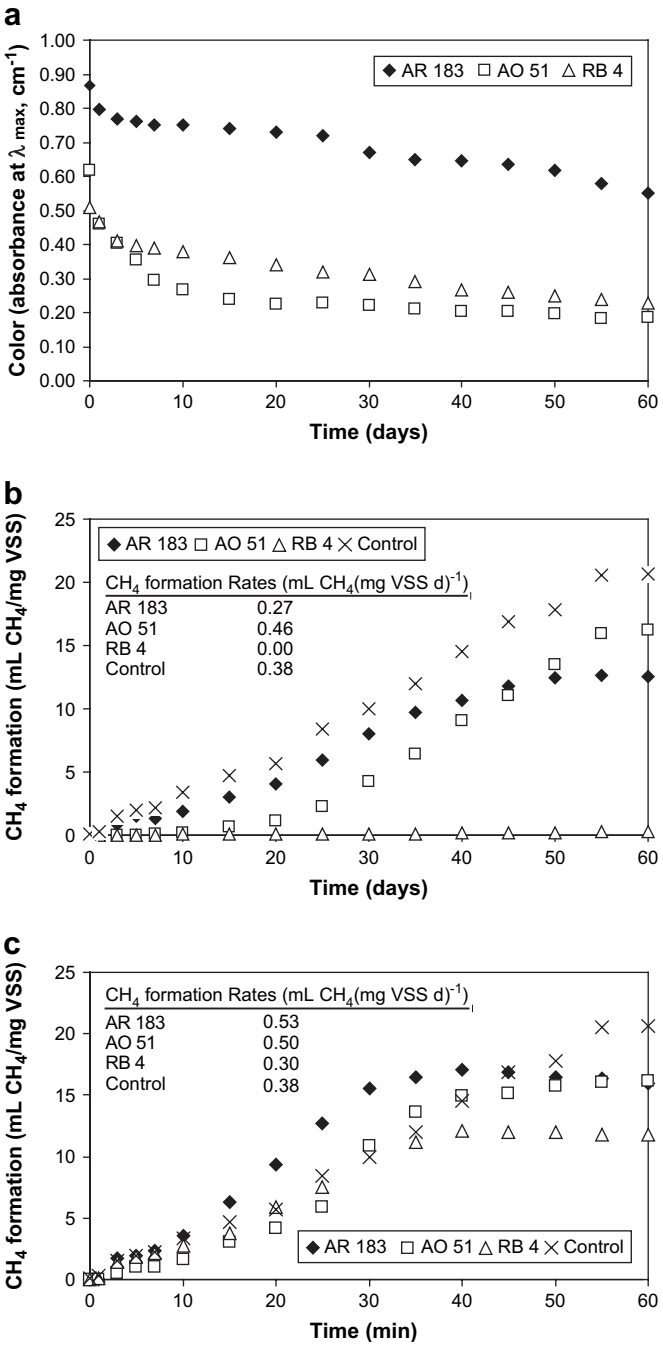


Fig. 4. (a) Color abatement and (b) methane production during anaerobic experiments with untreated and (c) Fenton-treated AR 183, AO 51 and RB 5.

dye (>50 mg/L), the use of an external carbon source was not necessary, since decolorization efficiencies as high as 98% were achieved after 72 h of treatment. However, when the concentration was higher than 50 mg/L, the use of a co-substrate (114 mg/L sodium acetate) was required to achieve the same treatment efficiency [43]. Compared with azo dyes, anthraquinone dyes are less susceptible to reduction and present very low color abatement capacity [49,50]. In an experiment that was conducted at an initial concentration of 300 mg/L of the anthraquinone dye Reactive Blue 4 to observe anaerobic color abatement, 78% decolorization was obtained after 28 days in the presence of 8400 mg/L VSS and an external carbon source mixture of 750 mg/L dextrine + 375 mg/L peptone. In the same study, decolorization of 300 mg/L Reactive Blue 19 (another anthraquinone dye) was 83% after 28 days [51]. These experimental findings were attributed to reductive transformation of the dyes [37]. Contrarily, in another study, 68 and 57% color removals were obtained for 20 mg/L Reactive Black 5 (a disazo dye) and Reactive Blue 19, respectively, after an incubation period of 16 days and in the presence of 1000 mg/L COD serving as the external carbon source. Decolorization was referred to sorption on bacterial flock material [48]. Generally speaking, when no readily biodegradable co-substrate is provided and no acclimation to the complex substrate is carried out, anaerobic decolorization can principally be ruled out. Even when the above given conditions are satisfied it is difficult to make a correct judgment about the dye removal mechanism since abiotic control experiments can be very misleading, the structure of the biosludge changes/deteriorates with biotreatment time leading to modified biomass surface properties [48]. Hence, in the present study it was assumed that color removal was mainly due to physical adsorption (biosorption onto bacterial cell walls).

3.3.2. CH_4 formation rates

Methane production was monitored to assess the effect of untreated and Fenton-treated dyes on anaerobic biomass (methanogenic activity). Anaerobic digestion is a multi-step process; methane production is the final, rate-limiting stage of anaerobic digestion and methane formation rate is a common tool to assess toxicity of industrial pollutants [2,49,52]. Fig. 4 presents methane production rates in the anaerobic bioreactors fed with untreated (b) and Fenton-treated (c) textile dyes. From Fig. 4(a)–(c) it is obvious that methane production rate is significantly faster in the presence of treated textile dyes than for the control sample, whereas the methane production rate is comparably slow for sludge digestion in the presence of untreated textile dyes. No methane production occurred in the sample bearing RB 4. This is in accordance with an investigation of Fontenot et al. [51] who studied the behavior of 500 mg/L aqueous Reactive Blue 4 solution under methanogenic conditions. For an incubation time of 15 days, only 6% CH_4 production (relative to the control) was observed in that experimental work for the sample with Reactive Blue 4. The inhibitory effect of untreated dyes on methane production can be explained by the occupation of the methanogenic bacteria's active sites by biosorbed dye molecules [49]. On the other hand, the Fenton-treated dyes had no inhibitory effect on methanogenesis except Fenton-treated RB 4. From Fig. 4(b) and (c) it is also interesting to observe that methane production rates started to level off at different days and that the control sample reached an asymptotic value appreciably later than the treated dyes. The presence of trivalent chromium in AR 183 might have had a toxic effect on methanogenic activity. Some related papers point out that heavy metal release from metal-complex dyes during biological and chemical treatment could be the major reason of the increase in acute toxicity. Osugi et al. [53] investigated the toxic effect of the copper–phthalocyanine dye Reactive Blue 15 on the photobacteria *Vibrio fischeri*.

Photoelectrocatalytic treatment caused a rapid increase in the acute toxicity of the dye that was explained by the release of elemental copper from the dye's structure at a rate of 55% (=0.85 mg/L) during its oxidation. In the present study, chromium levels in Fenton-treated AR 183 (supernatant) were determined as 1.2 mg/L and might probably be the reason for its inhibitory effect on methanogenic bacteria.

The methane production rate in samples containing untreated, treated dyes as well as control cultures could all be fitted to zero-order kinetics. The rate constants are provided in the inset of Fig. 4(b) and (c) and used to evaluate the relative inhibitory effect of the untreated and treated dyes in the forthcoming section.

3.4. Anoxic experiments

3.4.1. Color

Color abatement rates observed for the untreated textile dyes during anoxic experiments are presented in Fig. 5(a). Preliminary adsorption accounted for 62, 92 and 73% decolorization and total color removal was obtained as 72, 94 and 85%, revealing that the major fraction of absorbance was already removed before the denitrification process even started. Provided that the decolorization mechanism for AR 183 and AO 51 is reduction of the azo bonds, the low color abatement rates observed for the two azo dyes compared with RB 4 can be explained as the competition between NO_3^- and N and the azo dye for electron donors. This may be supported with the previous studies where color abatement under anoxic conditions was investigated. It was observed that NO_3^-–N acts as an electron acceptor and the presence of an alternative electron acceptor (namely an azo dye) may retard reductive decolorization [54–56]. Considering that the presence of nitrate could have hindered reductive azo bond cleavage of the two azo dyes but not the decolorization of the anthraquinone dye in the present study, it is not surprising that decolorization during denitrification was faster for RB 4. In another study carried out with 20 mg/L aqueous reactive dye solutions in the presence of 5 and 10 mM NO_3^-–N in a sequential anaerobic/aerobic reactor, the addition of nitrate had no adverse effect on color removal for the anthraquinone dyes because these dyes were removed via adsorption and not microbial degradation [56]. However, it should be kept in mind that in the present study the decolorization mechanism for the azo dyes AR 183 and AO 51 could also be biosorption. Hence the above explanation is only valid if decolorization is a consequence of azo bond reduction for azo dyes and biosorption for anthraquinone dyes. Considering that preliminary adsorption accounted for most of the color removal and the biomass in all anoxic reactors were colored after the experiments were completed, there is definitely more evidence that under our experimental conditions physical adsorption onto biomass (biosorption) played a major role in color removal of the selected textile dyes.

3.4.2. Denitrification rates

Fig. 5 displays denitrification rates observed during anoxic experiments in untreated (b) and Fenton-treated (c) samples as well as corresponding controls. As is evident in Fig. 5(b) and (c), nitrate concentration fell from 112 mg/L at the beginning of the anoxic experiment to 0–7.2 mg/L within the first 30 min in all bioreactors. Nitrate was completely consumed after 2 h in all bioreactors and initial denitrification rates were similar to each other (see inset in Fig. 5(b) and (c) for the initial reaction rate constants); from the denitrification rate

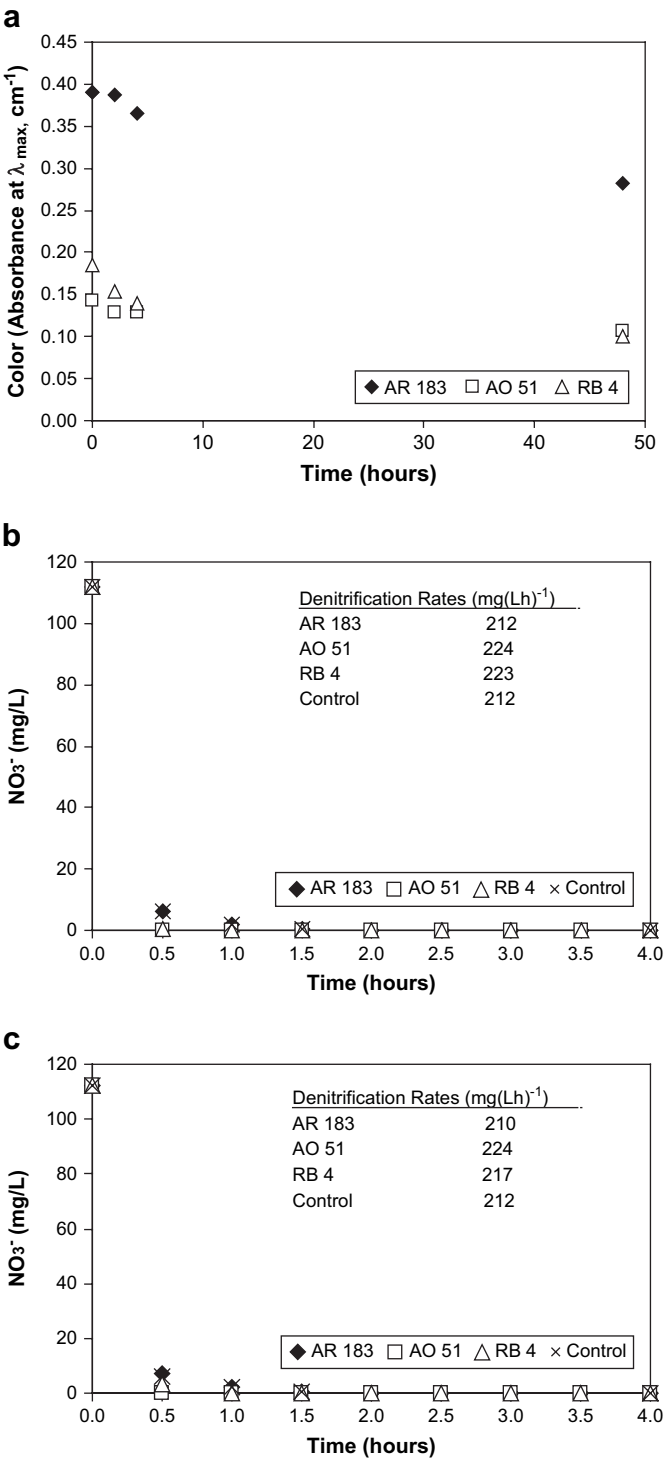


Fig. 5. (a) Color abatement and (b) denitrification during anoxic experiments with untreated and (c) Fenton-treated AR 183, AO 51 and RB 5.

constants it is clear that the untreated as well as Fenton-treated dyes did not adversely affect anoxic processes.

3.5. Aerobic experiments

3.5.1. Color

Fig. 6(a) depicts changes in color during aerobic treatment of the selected textile dyes. Again, preliminary adsorption onto biomass was also examined (not shown data) and found as 6, 93 and 41%, whereas overall color removal efficiencies were obtained as 22, 96 and 84% for AR 183, AO 51 and RB 4, respectively. It is known that azo-, nitro-, or sulfo-substitutions are not readily biodegradable under aerobic conditions [57]. Hence in the present study, color removal is expected to be a consequence of biosorption onto activated sludge. Buitron et al. [58] investigated the aerobic degradation of 25 and 50 mg/L Acid Red 151 solution in a sequencing batch biofilter packed with porous volcanic rock. In this stuy, Acid Red 151 was used as the sole carbon and energy source for microorganisms. After 24 h, 50% removal of the initial absorbance was observed; however, the removal mechanism was adsorption (onto the packing material) and biosorption (onto attached microorganisms). Pourbabaee et al. [59] studied the decolorization of 300 mg/L Terasil Black (a fiber disperse dye) under aerobic conditions in the presence of an exogenous carbon source (e.g. glucose and yeast extract). Decolorization of the effluent containing Terasil Black dye depended upon the presence of external carbon and energy sources. Nearly 60% of the original color disappeared after 30 h of incubation at a temperature of 30 °C in the presence of 10 g/L glucose and 5 g/L yeast extract, whereas only 10% decolorization occurred in their absence. Disperse dyes usually agglomerate fast and tend to precipitate out of the solution bulk easily. In our study, activated sludge biomass preferred to consume the easily degradable carbon source (glucose) instead of the carbon being incorporated in the complex dye molecules.

3.5.2. Glucose degradation rates

Fig. 6(b) shows glucose-COD degradation rates for untreated textile dyes and the control sample (operating conditions: VSS = 4400 mg/L; F/M = 0.12 mg COD_o/mg VSS). From Fig. 6(b) is can be seen that glucose degradation was practically complete within 30 min due to glucose exhaustion for all studied samples. The final CODs that were reached after 6 h aerobic biotreatment ranged between 37 mg/L (control) and 65 mg/L (sample containing glucose plus AR 183); the residual CODs were thought to be metabolic end products of glucose plus textile dyes that were not biosorbed onto activated sludge. This result also explains why the remaining COD was slightly higher for the sample bearing AR 183, which was at least sorbed onto aerobic cell biomass. Fig. 6(c) gives glucose-COD degradation rates for Fenton-treated textile dyes and the corresponding control sample (VSS = 3700 mg/L; F/M = 0.13 mg COD_o/mg VSS). Compared to the COD abatement rates shown in Fig. 6(b), those given in Fig. 6(c) were significantly slower because the VSS concentration in the samples bearing Fenton-treated dyes

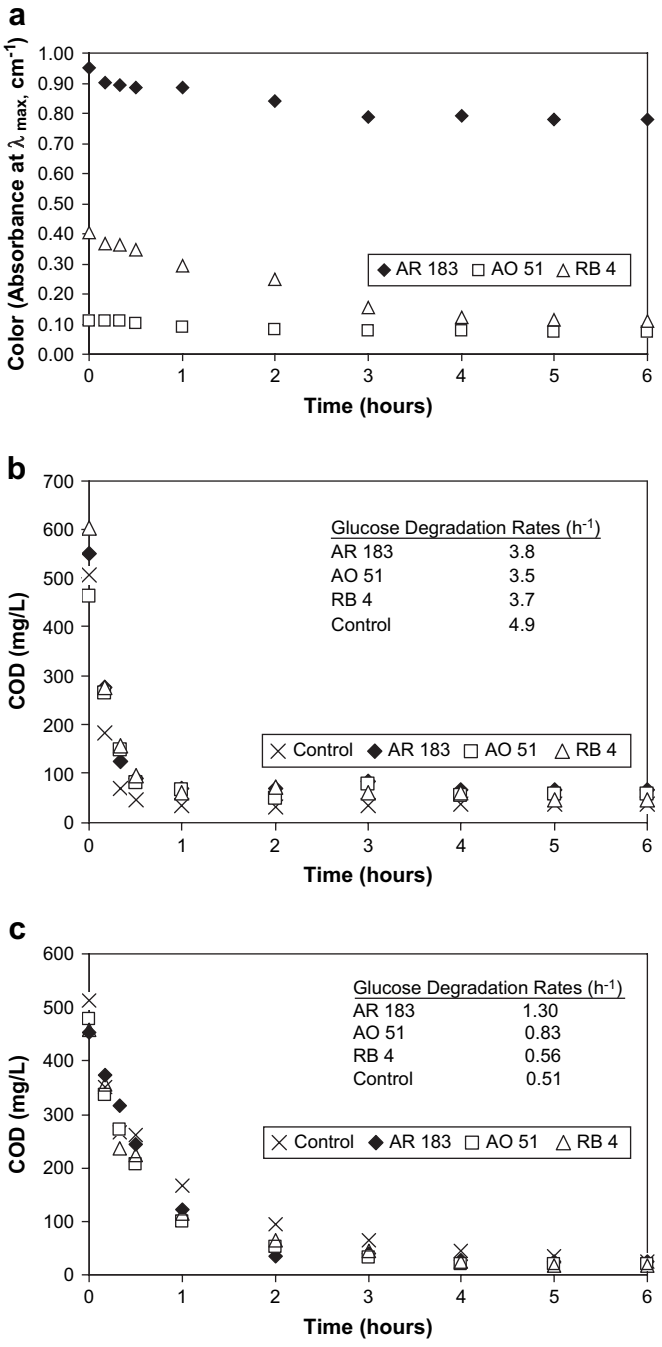


Fig. 6. (a) Color abatement and (b) glucose-COD degradation during aerobic experiments with untreated and (c) Fenton-treated AR 183, AO 51 and RB 5.

was a bit lower than that in the samples containing untreated dyes (3700 mg/L in the samples with treated dyes instead of 4400 mg/L in the sample with untreated dyes) that directly affected the F/M ratio and hence COD abatement rates. However, it should be emphasized that the COD fell down to a range of 17 mg/L (sample with treated RB 4) and 25 mg/L (control and sample with treated AR 183) implying that glucose degradation was more complete in the presence of Fenton-treated dyes. In all cases, glucose-COD abatement rates followed pseudo-first order kinetics (R^2 of the kinetic fit > 0.95) with respect to COD and the corresponding reaction

Table 4
Percent inhibition of CH_4 formation (I_{CH_4}), denitrification ($I_{NO_3^-}$) and glucose-COD (I_{COD}) abatement rates calculated for untreated and Fenton pre-treated (reaction conditions: $Fe^{2+} = 4$ mM; $H_2O_2 = 20$ mM; $pH_o = 3$; $t = 10$ min) AR 183, AO 51 and RB 4 (100 mg/L aqueous textile dye solutions) relative to the corresponding control experiments

Textile dye	Anaerobic I_{CH_4} (%)[a]		Anoxic $I_{NO_3^-}$ (%)[a]		Aerobic I_{COD} (%)[a]	
	Untreated	Treated	Untreated	Treated	Untreated	Treated
AR 183	29	0	0	0	23	0
AO 51	0	0	0	0	29	0
RB 4	100	21	0	0	25	0

[a] Percent inhibition was calculated as follows: I (%) = (methane formation or denitrification or glucose degradation rate constant in the control sample − methane formation or denitrification or glucose degradation rate constant in the textile dye sample) × 100/(methane formation or denitrification or glucose degradation rate constant in the control sample).

rate coefficients are listed in the inset of Fig. 6(b) and (c). From the rate constants it can be concluded that untreated textile dyes had a slightly inhibitory and the Fenton-treated dyes a significantly accelerating (providing more degradable substrate to the bioreactor) effect on glucose degradation with activated sludge.

3.6. Evaluation of the inhibitory effect on anaerobic, anoxic and aerobic treatment processes

Table 4 summarizes percent inhibition (I, %) of the methane production (anaerobic experiments), denitrification (anoxic experiments) and glucose degradation (aerobic experiments) rates obtained for untreated and Fenton-treated textile dyes AR 183, AO 51 and RB 4. The percent inhibition values were calculated on the basis of the reaction rate constants derived from the anaerobic (see Fig. 4), anoxic (Fig. 5) and aerobic (Fig. 6) experiments relative to the corresponding control reactors. From Table 4 it can be inferred that treated dyes exhibited no or appreciably less inhibitory effect on biomass than the untreated dyes implying that Fenton oxidation is an ecotoxicologically safe treatment option and can potentially be applied as a pre-treatment stage for fast/complete color and partial organic carbon removal prior to anaerobic, anoxic and aerobic processes.

4. Conclusions and recommendations

Textile dyes are known to resist conventional physicochemical (adsorption, coagulation) and aerobic biological treatment due to their high degree of polarity and complex molecular structure. As a consequence, textile dyes tend to sorb on activated sludge in aerobic biotreatment systems and on soil sediments in receiving water bodies, causing significant environmental, ecotoxicological and ecological problems. With these important issues in mind, the present study aimed at treating three commercially important textile dyes (Acid Red 183, AR 183; Acid Orange 51, AO 51; and Reactive Blue 4, RB 4) with Fenton's reagent to evaluate the effect of untreated and Fenton-treated textile dyes on aerobic, anoxic and anaerobic processes. Our results have demonstrated that during Fenton treatment of the selected dyes decolorization was complete within minutes and accompanied with appreciable COD and DOC removals. Acetate was detected as the major stable oxidation product and its concentration depended upon the dye type. Nitrate was expected due to the amino, nitro, azo, and triazynyl content of the investigated textile dyes, but did not form during Fenton treatment at significant levels (its concentration remained at the detection limit of the analytical equipment). Our experimental findings have also indicated that textile dyes have less inhibitory effect on anaerobic (methanogenesis) and aerobic (glucose abatement) processes than the untreated (original) ones when subjected to Fenton treatment under optimized reaction conditions. The untreated and Fenton-treated textile dyes did not exert any inhibitory effect on denitrification. On the other hand, our study indicated that decolorization via biological processes was mainly due to sorption onto biomass and only efficient for the disazo dye AO 51. Nevertheless, Fenton process can be recommended for complete, oxidative color and partial organic carbon removal; a feasible solution to meet color discharge standards, reduce the organic load associated with the dyes in dyehouse effluent without causing any inhibitory effect on anaerobic, anoxic and aerobic processes.

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