Reduction of acute toxicity and genotoxicity of dye effluent using Fenton-coagulation process

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ABSTRACT

Dye wastewater exhibits significant ecotoxicity even though its physico-chemical parameters meet the discharge standards. In this work, the acute toxicity and genotoxicity of dye effluent were tested, and the Fenton-coagulation process was carried out to detoxify this dye effluent. The acute toxicity was evaluated according to the mortality rate of zebrafish, and genotoxicity was evaluated by micronucleus (MN) and comet assays. Removal of color and chemical oxygen demand (COD) was also investigated. The results indicated that the dye effluent showed strong acute toxicity and genotoxicity to zebrafish. After 4 h of treatment by Fenton-coagulation process, the dye effluent exhibited no significant acute toxicity and genotoxicity to zebrafish. In addition, its COD was less than 50 mg/L, which met the discharge standard. It demonstrates that Fenton-coagulation process can comprehensively reduce the acute toxicity and genotoxicity as well as the COD of the dye effluent.

Keywords: Acute toxicity; COD; Dye effluent; Fenton-coagulation; Genotoxicity

1. Introduction

Dye wastewater, containing high concentrations of unfixed dyes, salts and other organic compounds, is one of the major industrial water pollution sources in developing countries [1]. More than 1.6×10^9 m³ dye wastewater is generated annually in China [2]. Azo dyes are one of the most common pollutants in the dye wastewater. Moreover, they are recalcitrant to be degraded by conventional biological wastewater treatment methods. Azo dyes have attracted more attention on its intermediates, especially aromatic amines, produced under anaerobic conditions and cannot be entirely removed by aerobic biological process. Aromatic amines were considered to be more toxic than azo dyes for their carcinogenic effect to aquatic life [3-5]. Consequently, effective treatment processes needs to be applied in the treatment of dye wastewater. Different kinds of treatment processes have been applied to treat this wastewater [6-9]. Advanced oxidation processes (AOPs) are efficient methods to degrade refractory organic pollutants, since they can generate the powerful oxidant, $\bullet\text{OH}$ ($E^\circ = 2.8$ V), without selectivity for most organic compounds [10]. Among the AOPs, Fenton process has gained increasing attention with its simple equipment, mild operating conditions (room temperature and atmospheric pressure) and effective $\bullet\text{OH}$ generation [10, 11]. The Fenton reaction mechanism is that H_2O_2 decomposes catalytically by Fe^{2+} to form the $\bullet\text{OH}$ at acidic pH (Equation (1)), which can oxidize most organic matter into harmless compounds such as CO_2 , H_2O , and some organic acids such as formic acid, acetic, and oxalic acids [11]. Additionally, Fe^{3+} can catalyze the H_2O_2 in the so-called Fenton-like reaction, regenerating Fe^{2+} , thus the process is sustaining via Equations (2)-(8) [12].

Both Fe^{2+} and Fe^{3+} ions are coagulants, simultaneous occurrence of oxidation and coagulation, can be obtained by adjusting pH in Fenton-coagulation process. It can achieve better removal of target compounds, color, and COD, than the Fenton process [10, 13, 14].

Little attention has been paid to assess the ecotoxicity of biologically treated dye effluent, when the chemical parameters of it meet the standard. Previous study has shown that despite of the reduction of color, COD and TOC, the wastewater ecotoxicity cannot be significantly reduced, even can be potentially increased [15-17]. Therefore, it is essential to evaluate dye effluent ecotoxicity for their potential risks to ecosystems. Acute toxicity is frequently used to reflect the ecotoxicity of wastewater to organisms exposed in them [18, 19]. Besides, the genotoxicity is of special concern, because it may cause adverse reproductive damage to organisms directly or even lead to their extinction [7, 20]. These two kinds of biological monitoring combined together can provide a relative comprehensive evaluation of the toxic hazard of wastewater on natural environments.

In this work, we assessed the chemical parameters and acute toxicity and genotoxicity of effluent from an industrial dye wastewater treatment plant (WwTP), and investigated the detoxification efficiency of Fenton-coagulation process in this real dye effluent treatment. Combination of chemical and biological analysis can provide comprehensive evaluation of dye effluent in order to avoid potential ecological risks. Furthermore, Fenton-coagulation process has been proven to be effective on detoxification of real dye effluent.

2. Materials and methods

2.1. Synthetic dye effluent

The active red azo dye 3BS and sucrose were dissolved in tap-water to prepared the synthetic effluent. The chemical formula of dye 3BS is shown in Fig.1 [21]. The final concentrations of dye and COD in synthetic dye effluent were 10 mg/L (9.21×10^{-3} mM) and 100 mg/L, respectively, which are close to those of real industrial dye effluent.

2.2. Real dye effluent

According to 24-h composite sampling method [22], the real dye effluent samples were collected from an industrial wastewater treatment plant of the textile factory (Liaoning, China) used dye 3BS as original materials. Samples were transported to the laboratory immediately and kept at 4 °C without any chemicals addition before analysis. Water quality of real dye effluent is given in Table 1.

2.3. Fenton process

The Fenton process was carried out in 5-L glass bottles at room temperature and atmospheric pressure. The initial pH of the sample was manually adjusted to 3 with 1 M H₂SO₄. Then the Fenton reagent was added in the reactor. The Fe²⁺ dose was varied between 0.18 to 0.9 mM. H₂O₂ was varied between 1.08 mM and 8.64 mM (0.18 to 1.5 times stoichiometric amount with respect to COD).

2.4. Coagulation process

Samples from each time interval of Fenton process were adjusted pH to 7 with 1 M NaOH for coagulation process.

2.5. Chemical analysis

COD, biological oxygen demand (BOD), NH₄⁺-N, suspended solid (SS), total nitrogen (TN) and total phosphorus (TP) were determined according to the APHA standard methods [23]. The pH was measured by a pH analyzer. TOC was determined by a TOC analyzer (Shimadzu, TOC-V_{CPH}, Japan). The color level was measured by the dilution times method [24]. The concentration of 3BS was measured by UV-vis

spectrophotometer (Techcomp, UV-2301, China) at 541 nm [25]. H₂O₂ concentration was determined by a spectrophotometric method as previously described [26].

2.6. Toxicity tests

2.6.1. Acute toxicity test

The 96 h acute-static toxicity test was done as previously described [27]. Zebrafish (*Tuebingen* strain, 3-6 months old) were exposed to different dilution volume ratios of effluent samples (5%, 10%, 25%, 50%, 75% and 100%) in triplicate. Potassium dichromate (> 30 mg/L) and 4-nitroquinoline-1-oxide (4-NQO) (> 60 mg/L) were set as positive controls.

2.6.2. Genotoxicity assays

The genotoxicity was assessed by MN and comet assays upon the zebrafish exposed to effluent samples for 96 h. Potassium dichromate (2.5, 5, 10 and 20 mg/L) and 4-NQO (20, 30, 40 and 50 mg/L) were set as positive controls (Fig. S1 and S2), meanwhile, uncontaminated dechlorinated tap water was set as negative control (NC). The dechlorinated tap water treated by Fenton-coagulation process was set as Fenton-coagulation process control (FC).

MN assay: Zebrafish blood was smeared on clean slides containing fetal bovine serum by making a caudal cut. Slides were air-dried, fixed in methanol and then stained in 10% Giemsa's stain. MN frequency was observed in the light microscope under oil immersion (10 × 100 magnification) [15].

Comet assay: Liver cells of zebrafish were used for the comet assay. Rough microscope slides were first coated with 1% normal melting point agarose (NMP) and solidified on ice. The supportive (second) agarose layer covered with 0.7% low melting point (LMP) agarose at a ratio of 1 part cells to 3 parts agarose to prevent the nuclear DNA from escaping during cell lysis and electrophoresis. The lysis of cells was carried out by incubating the microgels for 1.5 h at 4 °C in fresh lysis solution. The microgels were then submerged into a 4 °C alkaline electrophoresis buffer (300 mM NaOH, 1 mM EDTA, pH 13) for 20 min to unwind the nuclear DNA. Afterwards

the microgels were placed side by side into an electrophoresis chamber containing an electrophoresis buffer. Electrophoresis was performed at 25 V, 300 mA for 20 min. Following electrophoresis, the microgels were neutralized in a freshly prepared 400 mM Tris-HCl buffer (pH 7.5) for 15 min for three times. The DNA was stained with Gelred (0.2 $\mu\text{L}/\text{mL}$) before microscope analysis. Tail moment was used as the measure of DNA damage [15].

2.7. Statistical analysis

Tail moment was quantified using the image analysis program Casp 1.2.2 (Zbigniew Koza, Poland). The results were expressed as the mean $\pm$ SD. Statistically significant differences among means were determined by one-way analysis of variance (ANOVA) followed by Dunnett's test with significance at $p < 0.05$ for all tests.

3. Results and discussion

3.1. Application of Fenton-coagulation process on synthetic effluent

3.1.1. Color and COD removal of synthetic effluent by Fenton-coagulation process

Before treating the real dye effluent, the operation conditions and effectiveness of Fenton-coagulation process were investigated through treating the synthetic dye effluent. The effect of Fe^{2+} dose on 3BS and COD removal was investigated at the initial conditions: 4.32 mM H_2O_2 and $\text{pH} = 3$ (data not shown). The 3BS and COD removal efficiencies were increased when the Fe^{2+} dose increased from 0.18 mM, and reached maximum efficiencies (97% and 73%, respectively) at Fe^{2+} dose of 0.36 mM, and then declined when the dose of Fe^{2+} got higher than 0.36 mM. In subsequent studies, the initial Fe^{2+} dose was selected at 0.36 mM.

H_2O_2 plays an important role as an oxidant in the Fenton reaction for generating the $\bullet\text{OH}$. To optimize the dose of H_2O_2 in Fenton process, Fenton reagent ratio ($\text{M}[\text{H}_2\text{O}_2]:\text{M}[\text{Fe}^{2+}]$) from 3:1 to 24:1 (1.08 mM to 8.64 mM H_2O_2) under 0.36 mM Fe^{2+} at pH 3 was investigated. It showed that 97% of 3BS and 73% of COD could be

removed at the initial Fenton reagent ratio up to 12:1 (Fig. 2) since the presence of enough H_2O_2 catalyzed by Fe^{2+} for producing more $\bullet\text{OH}$ (Equation 1). However, decrements of 3BS and COD removal were observed when the Fenton reagent ratio was higher than 12:1 (data not shown), since the redundant H_2O_2 might scavenge $\bullet\text{OH}$ (Equation 5).

Then the coagulation process after Fenton process was investigated on the synthetic effluent. The 3BS and COD removal efficiencies were increased up to 99% and 80% by this process, respectively. The reason was that the formation of iron hydroxides, which could adsorb organic matters in the solution, led to the decrease of 3BS and COD [28].

3.1.2. H_2O_2 decomposition in Fenton and Fenton-coagulation processes

In Fenton and Fenton-coagulation processes, H_2O_2 may appear as a residue at the end of treatment and contribute to relatively higher toxicity than that of Fe^{2+} [29, 30]. Hence, the dose of H_2O_2 during the treatment was investigated as shown in Fig. 3. At the first stage of the reaction, the consumption rate of H_2O_2 was fast with the main reaction catalyzed by Fe^{2+} . Then the consumption rate of H_2O_2 got slow with the main reaction catalyzed by Fe^{3+} [11]. The H_2O_2 consumption was related to COD and 3BS removal in the synthetic effluent since $\bullet\text{OH}$ from H_2O_2 catalyzed by Fe^{2+} could oxidize the organic compounds. The coagulation process also reduced the H_2O_2 concentration, probably due to the increment of pH enhancing the decomposition of H_2O_2 into O_2 and H_2O as well as the adsorption of H_2O_2 on ferric hydroxide gel.

3.1.3. Acute toxicity of the synthetic effluent

To clarify the potential risk of synthetic effluent during Fenton-coagulation process, the evolution of acute toxicity was investigated (Table 2). The NC and FC did not pose any acute toxicity to zebrafish. The acute toxicity of synthetic effluent showed specific dose-dependence. The Fenton-coagulation process resulted in the augmentation of acute toxic effects on zebrafish at initial periods of the process. At Fenton reagent ratio of 3:1, the mortality rate increased from 8% to about 23% after

0.5 h treatment, and was not reduced by extending treatment time. However, at Fenton reagent ratio of 6:1 and 12:1, the acute toxicity, increasing to 65% and 25% after 0.5 h treatment, was removed by extending treatment time. It implied that the formation of intermediate products was more toxic than their parent compounds, and could be degraded by Fenton-coagulation process in enough reaction time at the appropriate Fenton reagent ratio. Moreover, a faster degradation rate was achieved at Fenton reagent ratio of 12:1 than that of 6:1 because of higher amount of $\bullet\text{OH}$ produced. Therefore, the acute toxicity removal of synthetic effluent depended on the Fenton reagent ratio. Furthermore, the coagulation process also promoted the acute toxicity removal through the sedimentation of toxic pollutants.

3.1.4. Genotoxicity of the synthetic effluent

Dye effluent may bring potential damage to the chromosome and DNA of organisms. MN and comet assays were used to characterize the genotoxicity of synthetic dye effluent after Fenton-coagulation process, and the results were shown in [Fig. 4](#). The genotoxicity of FC at each interval was not significantly different from that of NC, thus residues of Fenton-coagulation process do not contribute to the genotoxicity. All the synthetic effluent samples before and during Fenton-coagulation process did not exhibit any acute toxicity at 25% dilution while some of them displayed significant acute toxicity without dilution. So in the genotoxicity removal experiments, the genotoxicity of the synthetic effluent samples were performed at 25% dilution to avoid the interference by the acute toxicity. The results showed that the raw synthetic effluent induced a strong genotoxicity response toward zebrafish assessed by both MN and comet assays. The effluent treated by Fenton-coagulation process at all three Fenton reagent ratios caused significant increase in genotoxicity at the initial stage of process. However, the genotoxicity decreased during the treatment at Fenton reagent ratio of 6:1 and 12:1, achieving 46% and 79% reduction of MN frequency, and 30% and 77% reduction of tail moment, except for the samples treated at Fenton reagent ratio of 3:1, which presented no decrease in the genotoxicity for

even 4 h treatment. The genotoxicity evolution was probably due to the rapid transformation of compounds in synthetic effluent into more toxic intermediates and the subsequent degradation of the intermediates into inorganic compounds and organic acids with low genotoxicity [31]. Increasing initial Fenton reagent ratio enhanced the genotoxicity reduction with the formation of more $\bullet\text{OH}$ that could oxidize the synthetic effluent and its intermediates. In addition, the Fenton-coagulation process could achieve a better detoxification by oxidation and sedimentation of the toxic contaminants than Fenton process.

3.2. Application of Fenton-coagulation process on real effluent

3.2.1. COD evolution of real effluent

According to Chinese Sewage Discharge Standard (GB4287-2012), only color, BOD, SS and pH parameters are regulated. The levels of COD, TP, TN and $\text{NH}_4^+\text{-N}$ in real effluent are beyond the discharge standards as shown in Table 1. The BOD/COD ratio of real dye effluent (< 0.3) was rather low implying its low biodegradability. Therefore, Fenton-coagulation as an alternative advanced treatment process was attempted to treat this type of effluent aiming to meet the discharge standard.

From the part of 3.1., it showed that the acute toxicity and genotoxicity of synthetic effluent could not be removed and even increased at low Fenton reagent ratio of 3:1 (Table 2 and Fig. 4). Here, the initial conditions of Fenton-coagulation process were 0.36 mM Fe^{2+} , $\text{pH} = 3$, and Fenton reagent ratios varying at 6:1 and 12:1 (Fig. 5). The results indicated that increasing the Fenton reagent ratio from 6:1 to 12:1 could improve COD removal. At Fenton reagent ratio of 6:1, 38% and 56% COD removal were achieved in Fenton and Fenton-coagulation process, respectively, while at Fenton reagent ratio of 12:1, 51% and 65% COD could be removed in Fenton and Fenton-coagulation process, respectively. The final COD of real effluent all met the Chinese Sewage Discharge Standard ($< 50 \text{ mg/L}$), except that of the effluent treated by Fenton process at Fenton reagent ratio of 6:1. In Fenton process, the dose of Fenton reagent was in direct proportion to the COD removal. In coagulation process,

COD removal could be further increased by the emergence of iron flocs deposits. The COD removal of real effluent treated by Fenton-coagulation process was much lower than that of synthetic effluent. It probably resulted from undesirable side reactions that occurred between the $\bullet\text{OH}$ and anions present in the real effluent, such as chlorides, carbonates, sulfates and so on [32].

3.2.2. H_2O_2 decomposition during the Fenton and Fenton-coagulation processes

The H_2O_2 concentration in the real effluent treated at Fenton reagent ratios of 6:1 and 12:1 by Fenton and Fenton-coagulation process were measured (Fig. 6). It decreased continually until completely exhausted. The consumption of H_2O_2 was related to the reaction of H_2O_2 and Fe^{2+} , producing $\bullet\text{OH}$ and consequently achieving a high efficiency of COD removal as shown in Fig. 5. Subsequently, the coagulation process also promoted the H_2O_2 consumption for the adsorption of H_2O_2 on the oxyhydroxides formed by Fe^{3+} precipitation.

3.2.3. Acute toxicity of real effluent

In some cases, the COD removal does not show significant toxicity reduction after advanced treatments, or the intermediates cause more toxicity than their parent compounds [33-37], so it is necessary to carry out further toxicity tests. The real effluent from the dye WwTP showed acute toxicity to zebrafish with mortality rate of about 17% (Table 3). At Fenton reagent ratio of 6:1, the mortality rate of zebrafish was increased to 30% when the real effluent was treated by Fenton-coagulation process for 1 h. With extending treatment time, a fast decrease of acute toxicity was observed. The increased acute toxicity at initial stage was probably due to the production of intermediates with more toxicity to zebrafish as literature reported [38]. The acute toxicity decreased rapidly at Fenton reagent ratio of 12:1, achieving no inhibition of zebrafish survival after 1 h treatment. It was implied that more $\bullet\text{OH}$ generated attacked the organic matters through the reaction of Fe^{2+} and H_2O_2 (Equation 1).

3.2.4. Genotoxicity of real effluent

Table 3 demonstrated that effluent exhibited significant acute toxicity without dilution while it had no acute toxicity with 75% dilution. Accordingly, the genotoxicity of the real effluent were tested at 75% dilution for avoiding the interference of acute toxicity. The genotoxicity of real effluent before and after Fenton-coagulation process was tested as shown in Fig. 7. The raw real effluent sample without treatment induced high genotoxicity for the zebrafish. Based on the MN assay (Fig. 7 (a)), at Fenton reagent ratio of 6:1, the genotoxicity of real effluent increased in the initial 1 h and only 41% of genotoxicity was reduced after 4 h treatment by Fenton-coagulation process. At Fenton reagent ratio of 12:1, the rising tendency of genotoxicity in the initial 1 h was also observed, however, the genotoxicity decreased fast and was not significantly different from NC after 4 h since more $\bullet\text{OH}$ was produced.

Based on the comet assay (Fig. 7 (b)), at Fenton reagent ratio of 6:1, the genotoxicity of the real effluent remained nearly unchanged during the initial 1 h treatment and was significantly removed from 1 to 4 h. At Fenton ratio of 12:1, the genotoxicity was reduced to almost the same level as NC after 4 h treatment. The genotoxicity removal efficiency was higher at the ratio of 12:1 than that of 6:1. Therefore, Fenton reagent ratio of 12:1 was the appropriate condition for genotoxicity removal.

The MN assay demonstrated that the genotoxicity of real effluent treated by Fenton-coagulation for 1 h was higher than that of raw real effluent; but the comet assay showed the contrary results on genotoxicity. In some cases, MN and comet assays achieved the different results on genotoxicity for the different mechanisms of these two bioassays. The MN assay is related to chromosome aberrations in a meiotic or mitotic division, such as losses [39]. The comet assay examines DNA strand breaks and alkali labile sites (single and double-strand breaks) by measuring the migration of DNA from immobilized nuclear DNA [40]. Besides, the MN and comet assays employed the different body tissues of zebrafish, peripheral blood and liver,

respectively. Therefore, both bioassays taken into account can give an overall evaluation of the potential genotoxicity.

The ecotoxicity data suggest it is not appropriate simply relying on chemical estimates in wastewater risk [41]. Chemical analyses combined with ecotoxicity bioassays can draw a comprehensive assessment of wastewater risk. It can provide a scientific basis for wastewater discharge standards and a scientific technology for detoxification.

4. Conclusions

The chemical parameters, acute toxicity and genotoxicity of synthetic and real dye effluent were investigated and detoxification of both kinds of effluent was achieved using Fenton-coagulation process. For synthetic effluent, 99% of color and 80% of COD were removed under the conditions of Fe^{2+} dose of 0.36 mM and Fenton reagent ratio of 12:1 at pH 3 after 4 h treatment, meanwhile, the acute toxicity was completely removed and the genotoxicity was reduced to almost the same level as NC. For real dye effluent, COD with removal efficiency up to 65% was 33.6 mg/L, which met the Chinese Sewage Discharge Standard. The acute toxicity was completely removed, and the genotoxicity was reduced to the same level as NC and FC after 4 h of treatment. Taking together, this work demonstrated that Fenton-coagulation process can provide comprehensive treatment for mineralization and detoxification of dye effluent. Moreover, this study also illustrates the essential need for a battery of bioassays in ecological risk assessment of effluent.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at

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Figure captions:

Fig. 1. Chemical formula of dye 3BS.

Fig. 2. Effects of Fenton reagent ratio on synthetic effluent treatment by Fenton and Fenton-coagulation processes: (a) degradation of 3BS, (b) removal of COD. $\text{pH}_0 = 3$, $[\text{Fe}^{2+}]_0 = 0.36 \text{ mM}$, $C_{0,3\text{BS}} = 10 \text{ mg/L}$, $\text{COD}_0 = 100 \text{ mg/L}$.

Fig. 3. Decomposition of H_2O_2 in the synthetic effluent during the Fenton and Fenton-coagulation processes at different Fenton reagent ratios. $\text{pH}_0 = 3$, $[\text{Fe}^{2+}]_0 = 0.36 \text{ mM}$, $C_{0,3\text{BS}} = 10 \text{ mg/L}$, $\text{COD}_0 = 100 \text{ mg/L}$.

Fig. 4. Genotoxicity bioassays of 25% diluted synthetic effluent during the Fenton-coagulation process at different Fenton reagent ratios (3:1, 6:1 and 12:1) (a) MN assay, (b) comet assay. $\text{pH}_0 = 3$, $[\text{Fe}^{2+}]_0 = 0.36 \text{ mM}$, $C_{0,3\text{BS}} = 10 \text{ mg/L}$, $\text{COD}_0 = 100 \text{ mg/L}$, $\text{pH}_t = 7$. * Statistically significant differences according to NC ($p < 0.05$).

Fig. 5. Evolution of COD for different Fenton reagent ratios on real effluent treatment during Fenton and Fenton-coagulation processes. $\text{pH}_0 = 3$, $[\text{Fe}^{2+}]_0 = 0.36 \text{ mM}$, $\text{COD}_0 = 96.1 \text{ mg/L}$.

Fig. 6. Decomposition of H_2O_2 in the real effluent during the Fenton and Fenton-coagulation processes at different Fenton reagent ratios. $\text{pH}_0 = 3$, $[\text{Fe}^{2+}]_0 = 0.36 \text{ mM}$, $\text{COD}_0 = 96.1 \text{ mg/L}$.

Fig. 7. Genotoxicity bioassays of 75% diluted real effluent during the Fenton-coagulation process for different Fenton reagent ratios (6:1 and 12:1), (a) MN assay, (b) comet assay. $\text{pH}_0 = 3$, $[\text{Fe}^{2+}] = 0.36 \text{ mM}$, $\text{COD}_0 = 96.1 \text{ mg/L}$, $\text{pH}_t = 7$. * Statistically significant differences according to NC ($p < 0.05$).

Table legends:

Table 1. Water quality of the real dye effluent.

Table 2. Acute toxicity of synthetic effluent during the Fenton-coagulation process at different Fenton reagent ratios ($\text{pH}_0 = 3$, $[\text{Fe}^{2+}]_0 = 0.36 \text{ mM}$, $C_{0, \text{3BS}} = 10 \text{ mg/L}$, $\text{COD}_0 = 100 \text{ mg/L}$, $\text{pH}_t = 7$). Both NC and Fenton reagent control with 100% survival

Table 3. Acute toxicity of real effluent during the Fenton-coagulation process at different Fenton reagent ratios ($\text{pH}_0 = 3$, $[\text{Fe}^{2+}]_0 = 0.36 \text{ mM}$, $\text{COD}_0 = 96.1 \text{ mg/L}$, $\text{pH}_t = 7$). Both NC and Fenton reagent control with 100% survival

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Table 1. Water quality of the real dye effluent.

Parameter	Unit	Effluent	Maximum allowable value
pH	-	7.5	6-9
Color	dilution times	30	70
COD	mg/L	96.1	50
BOD	mg/L	20	25
BOD/COD ratio	-	0.21	-
SS	mg/L	53.4	60
TP	mg/L	1.1	1.0
TN	mg/L	67.7	20
TOC	mg/L	20.6	-
NH ₄ ⁺ -N	mg/L	61.3	12

- means not exist

Table 2. Acute toxicity of synthetic effluent during the Fenton-coagulation process at different Fenton reagent ratio ($\text{pH}_0 = 3$, $[\text{Fe}^{2+}]_0 = 0.36 \text{ mM}$, $\text{C}_{0, \text{3BS}} = 10 \text{ mg/L}$, $\text{COD}_0 = 100 \text{ mg/L}$, $\text{pH}_t = 7$). Both NC and Fenton reagent control with 100% survival

Treatment time (h)	Dilution (%)	Mortality rate (%) (Fenton reagent ratio)		
		3:1	6:1	12:1
0	100	8±5	8±5	8±5
0.5	100	23±5	65±6	25±6
	75	0	30±8	8±5
	50	0	8±5	0
	25	0	0	0
1	100	23±5	20±8	0
	75	0	0	0
2	100	23±5	0	0
	75	0	0	0
3	100	25±6	0	0
	75	0	0	0
4	100	26±5	0	0
	75	0	0	0

Table 3. Acute toxicity of real effluent during the Fenton-coagulation process at different Fenton reagent ratios ($\text{pH}_0 = 3$, $[\text{Fe}^{2+}]_0 = 0.36 \text{ mM}$, $\text{COD}_0 = 96.1 \text{ mg/L}$, $\text{pH}_t = 7$). Both NC and Fenton reagent control with 100% survival

Treatment procedure	Time (h)	Dilution (%)	Mortality rate (%)
Raw real wastewater	0	100	17 ± 6
		75	0
$[\text{H}_2\text{O}_2]:[\text{Fe}^{2+}] = 6:1$	1	100	30 ± 8
	2	100	0
	3	100	0
	4	100	0
$[\text{H}_2\text{O}_2]:[\text{Fe}^{2+}] = 12:1$	1	100	0
	2	100	0
	3	100	0
	4	100	0

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Figure 7