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Metal ion-catalyzed mineralization and biodetoxification studies of Calconcarboxylic acid in aqueous solution: effect of –COOH group

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ABSTRACT

Mineralization and biodetoxification studies of Calconcarboxylic acid (CCA), an azo dye, were undertaken by metal ion-catalyzed oxidation processes, viz. Fenton and Fenton-like processes and the effect of presence of –COOH group thereon were established. The metal ions used were Fe^{2+} and Ag^{+} and the oxidants used were hydrogen peroxide (HP) and ammonium persulfate (APS). For a given oxidant, Fe^{2+} is found to be the most suitable catalyst and among the oxidants, APS proves to be most suitable one. As all the systems yield maximum mineralization at pH 1, Fe^{2+}/APS at pH 1 is the most suitable system. It is observed that mineralization is favored by Fe^{2+}/HP process due to the presence of –COOH group in CCA and by Fe^{2+}/APS process in its absence. Removal of –COOH group causes a shift in optimum pH, for Fenton process, to 3 accompanied by a corresponding drop in mineralization from 70.6 to 35.6% and an increase from 73.2 to 91.1% in Fe^{2+}/APS system. Bioassay experiment reveals that the treated solutions are ≥50% biotoxic than the untreated dye, which could be reduced to 22.7 and 26.7% in Fe^{2+}/APS and Ag^{+}/APS systems, respectively, when the –COOH group is removed.

Keywords: Calconcarboxylic acid; Fenton and Fenton-like processes; Effect of –COOH group; Mineralization; Biodetoxification

1. Introduction

Advanced oxidation processes (AOPs) involving the oxidative degradation of organic pollutants by reactive hydroxyl and sulfate radicals have gained considerable importance in recent years due to their advantage over conventional methods of wastewater treatment [1]. Possessing high-oxidation potentials (2.65–2.80 V at pH 3.0 by $HO^{\bullet}$ and 2.5–3.1 V by $SO_4^{\bullet-}$ radical) [2], these radicals successfully attack the organic molecules and remove organic carbon present in them in the form of carbon dioxide, water, and other mineral salts in a process called mineralization. The hydroxyl and sulfate radicals are generated *in situ* by various methods e.g. photolysis by UV light in the presence of hydrogen peroxide (HP) and persulfate as oxidants (UV/HP or UV/persulphate); Fenton process using ferrous iron and an oxidant like HP (Fe^{2+}/HP), and a combination of both, in which case, it is called photo-Fenton process (Fe^{2+}/HP/UV). Fenton-like process is a modified form of Fenton process when persulfate is used as oxidant instead of HP or

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transition metal ions such as Co^{2+} and Ag^{+} ions are used instead of Fe^{2+} [3–5]. Fenton and Fenton-like processes are considered as attractive alternatives for wastewater treatment as iron is non-toxic and available abundantly in nature. Very recent applications of Fenton and Fenton-like processes are found in literature [6–9].

Azo dyes released from textile and dye stuff industries have much adverse impact on the biotic component of the environment. They prevent photosynthesis in aquatic plants and may have toxic or carcinogenic effect on aquatic organisms as well as on human beings. Therefore, proper treatment of wastewater containing azo dyes is necessary to completely mineralize them before releasing to the water bodies. Another important aspect of wastewater treatment is to ensure elimination of biotoxicity in the post-treated solution, so that the treated solution does not adversely affect the biotic component of the environment. In this study, Calconcarboxylic acid (CCA) is used as a model dye pollutant. This study utilizes the oxidizing ability of hydroxyl and sulfate radicals formed by the metal ion-catalyzed decomposition of two oxidants such as HP and ammonium persulfate (APS) for the decolorization and mineralization of CCA. The metal ions used in these processes (M^{n+}/oxidant), the so-called Fenton and Fenton-like processes, are Fe^{2+} and Ag^{+} ions. It is planned to find the most suitable operational parameters, e.g. pH, concentration of oxidant and metal ions for simultaneous mineralization and removal of biotoxicity (if any) in the post-treated solution. Recently, we have published papers on the mineralization of Calcon, an azo dye, by Fenton and Fenton-like processes [5] using HP and APS as oxidants and metal ions like Fe^{2+}, Ag^{+}, and Co^{2+}. The only structural difference between the two azo dyes, i.e. Calcon and CCA, is the presence of an additional –COOH at the C-3 position of CCA. It is, therefore, important to study the effect of removal of –COOH group on decolorization, mineralization of CCA, and the biotoxicity of the treated solutions by these processes. Since removal of –COOH group in CCA essentially gives Calcon; the study will provide a direct comparison between CCA and Calcon on their response to these processes towards decolorization, mineralization, and biotoxicity of the treated solutions. So, the results obtained in this study are compared with that of Calcon published earlier and should be referred to Sahoo et al. [5] *Escherichia coli* (*E. coli*) have been taken as the representative micro-organism for bioassay of the post-treated solutions. The biotoxicity assessment in this study is based on the metabolic inhibition (growth inhibition) bioassay of *E. coli*. As colony-forming unit (CFU) assay in a particular test solution is a measure of the reproductive ability of *E. coli*, its metabolic inhibition or growth inhibition bioassay can be measured by counting the CFU. CFU-based assessment has proved to be a reliable measure of biotoxicity [10] and has been applied in our earlier studies [5,11–13]. It is worth mentioning here that some authors have reported the disinfection of drinking water by AOPs including Fenton and Fenton-like processes by killing the micro-organisms [14,15]. On the other hand, we aim to generate effluents after the treatment where the micro-organisms can sustain their lives. Thus, the aim of both types of studies opposes each other. We emphasize that both studies are worth consideration. While the former is required for drinking water purposes, the later is required for conservation of eco-system. It must be mentioned here that killing of micro-organisms during disinfection of drinking water is desirable, while it is not in case of wastewater treatment as it would adversely affect the eco-system.

2. Materials and methods

2.1. Reagents and supplies

The azo dye, CCA (Synonyms: Cal-Red, Patton and Reeder's reagent; IUPAC name: 2-Hydroxy-1-(2-hydroxy-4-sulfo-1-naphthylazo)-3-naphthoic acid; molecular formula: $C_{21}H_{14}N_2O_7S$; Molecular weight: 438.414 ($g\,mol^{-1}$); λ_{max} = 550 nm), used in this work was purchased from Sigma Aldrich (Germany). Its

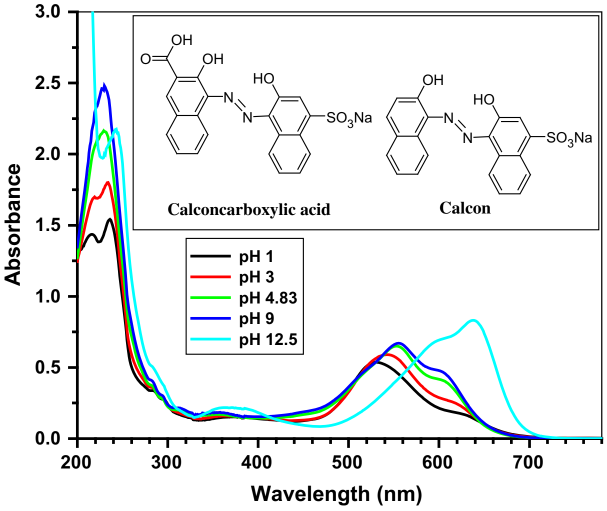

Fig. 1. UV–vis absorption spectra of CCA in aqueous solution at different pH values: [CCA] = 0.05 mM. Inset: Structures of Calcon and CCA.

molecular structure is shown in Fig. 1 (inset). Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$, GR), silver nitrate ($AgNO_3$, GR), HP (H_2O_2, 30% w/w, GR), sulphuric acid (H_2SO_4, GR), sodium hydroxide (NaOH, GR), and ammonium persulfate ($(NH_4)S_2O_8$, extra pure) used in this work were acquired from Merck (India). Luria Bertoni (LB) agar, LB broth, and ampicilin used in detoxification experiment were acquired from HiMedia (India). All the reagents were used without further purification.

2.2. Experimental procedure

Millipore water (Millipore India, Bangalore; Model: Elix3 Century) was used for preparing the CCA and other solutions throughout the experiment. The initial pH of the solution in each experiment was adjusted to the desired value with H_2SO_4 (the strength varying between 0.05 and 0.2 N depending on the pH required) or NaOH (the strength varying between 0.025 and0.1 N depending on the pH required) using a digital pH meter (Make: Chemiline; Model: CL 110). All the solutions used in this work were freshly prepared except the dye solution, which was stored at 4°C and used within three days. It may be mentioned that neither pH nor temperature was controlled during the course of the experiments.

The solutions obtained in all the treatment processes were used for analysis for decolorization and mineralization studies as well as biotoxicity assessment studies, as described subsequently. Decolorization studies were carried out by measuring the decrease in absorbance at 530 nm (at pH 1 and 3), 550 nm (at pH 4.83), 554 nm (at pH 9), and 638 nm (at pH 12.6) with the help of a UV–vis spectrophotometer (Perkin Elmer; Model: Lambda 25). The UV–vis absorption spectra of CCA in aqueous solution at different pH values are shown in Fig. 1. Mineralization studies were carried out by measuring the total organic carbon (TOC) of the samples with a TOC analyzer (Elementar Analysensysteme GmbH, Model: Liqui TOC II) using the procedure described before [16]. All the analyses were carried out immediately after the reaction and the reproducibility in TOC measurement was checked by frequent measurement of the standard (1:1 solution of potassium phthalate and sodium carbonate as TOC and total inorganic carbon standard, respectively) as suggested by the manufacturer, and the errors were always found to be within ±5%. The data presented in the text and figures were analyzed by standard deviation using “Origin 7” (Microcal Inc.) and has been rounded up to significant values.

2.2.1. Fenton and Fenton-like processes

All the reactions were carried out in the presence of air and at room temperature (varying between 21 and 23°C) by placing required volume of the dye solution of required concentration and pH in amber borosilicate reagent bottles. Each bottle was designated to be sacrificed for analysis after a predetermined time interval. The mouths of the bottles were covered with aluminum foil, and two holes were pierced through it to allow free passage of air. The reaction was initiated by adding 0.5 ml of required concentration of metal ion solution with constant stirring by a magnetic stirrer followed by the addition of 0.5 ml of required concentration of oxidant to the dye solution of desired pH. To 0.5 ml of 10 mM solution of each of different metal ions, 0.5 ml of 110 mM HP solution and 90 mM APS solution were added separately to the dye solution and, in all the cases, the total volume of the solution was kept constant at 100 ml. Therefore, the actual concentration of metal ions in the solutions was maintained at 0.05 mM and those of HP and APS at 0.55 and 0.45 mM, respectively. The reaction was continued for specified time period and then the resulting solution was analyzed as specified earlier.

2.2.2. Biotoxicity assessment of the treated solutions

Biotoxicity of the solutions after treatment was assessed by the method adopted by us and reported earlier [5,11]. Briefly, 40 g of LB agar dissolved in 1,000 ml of water was autoclaved for 30 min. Upon cooling down to about 40°C, Ampicilin (1 μl for each ml of LB agar solution) was added, mixed, and aliquots of approximately 10 ml of the medium were poured into sterilized Petri plates. LB broth (25 g) was separately dissolved in 1,000 ml of water, aliquoted (9 ml) in 100 ml conical flasks, and sterilized by autoclaving for 30 min. To the sterilized LB broth, 10 μl of ampicilin, 100 μl of the *E. coli* culture inocula, and 1 ml of the dye or other test solution were added and the cultures were grown at 37°C overnight in a rotary shaker. The following day, the mid-log phase culture was diluted 10,000 times and 10 μl of the diluted culture was spread over the LB agar plates prepared earlier. The plates were incubated for 24 h in a 37°C incubator as described earlier [17]. CFUs formed in each plate were counted. All the experiments were performed under sterilized conditions.

3. Results and discussion

3.1. *Effect of oxidant dosage*

The study in this section deals with mineralization of CCA by sulfate and hydroxyl radicals generated by various systems involving catalytic activity of metal ions on the oxidants viz. Fe^{2+}/APS, Fe^{2+}/HP, Ag^{+}/APS, and Ag^{+}/HP. The concentration of the oxidants has been proved to be critical in achieving maximum mineralization and hence, must be controlled. For this reason, we attempted first to optimize the concentration of oxidants keeping other operational parameters constant. This optimization is based on mineralization, although the trend in decolorization has been examined. It is observed that with increase in concentration of HP and APS in Fe^{2+}-catalyzed systems, both decolorization and mineralization increase until 0.55 mM of HP and 0.45 mM of APS, beyond which both starts decreasing. Thus, these values are considered to be the best suitable concentrations for the oxidants. The corresponding data are shown in Table 1 and graphically represented in Figs. 2 and 3. The decrease in decolorization and mineralization at higher concentration of oxidants may be due to the scavenging of sulfate and hydroxyl radicals. A similar result, obtained in our earlier study on Calcon, has been published by us recently and the reasons for the same have been discussed there in detail [5]. It is further noticed that both APS and HP decolorize the dye solution to the extent of ≥ 90% at their optimum concentrations in 240 min. On the other hand, during the same time period, both yield 78.3 and 74.1% mineralization, respectively, under similar conditions. Thus, the mineralization efficiencies of APS and HP are almost same. However, persulfate-based oxidation process has several advantages over HP such as (i) It has higher oxidation potential (E_0 = 2.01 V) than, H_2O_2 (E_0 = 1.76 V); (ii) It is solid at ambient temperature, easy to store and transport; (iii) $SO_4^{\bullet -}$ radicals are more stable than $HO^{\bullet}$ radicals; (iv) $S_2O_8^{2-}$ is practically inert and is not considered to be a pollutant. Another important advantage of using persulfate in oxidation process is that in aqueous solution, it generates hydroxyl radicals [18] and can participate in oxidation process (Eqs. (1) and (2)). This is the reason why APS system shows slightly higher mineralization efficiency than HP. Thus, we may conclude that APS is a better oxidant than HP. No separate optimization of oxidants was done for Ag^{+}-catalyzed systems. To evaluate the effect of equimolecular concentration of the oxidants in both the metal ion-catalyzed systems, we have considered 0.55 mM of HP and 0.45 mM of APS in Ag^{+}-catalyzed systems.

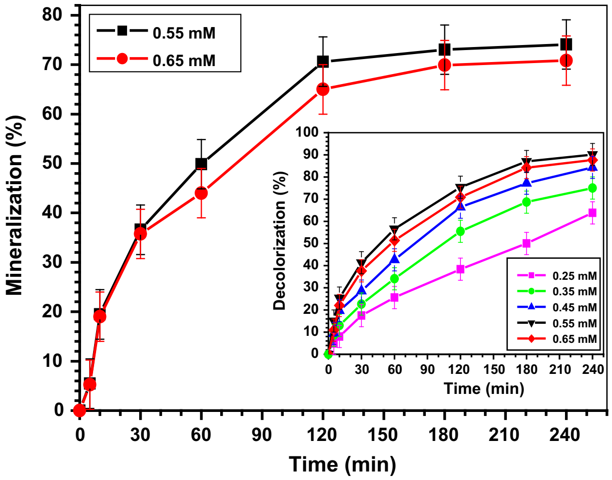

Fig. 2. Effect of HP concentration on the mineralization and decolorization (inset) of CCA: [CCA] = 0.05 mM; [Fe^{2+}] = 0.05 mM; pH 1.

Table 1
Effect of oxidant concentration on the decolorization and mineralization of CCA in Fe^{2+}/oxidant system: [CCA] = 0.05 mM; [Fe^{2+}] = 0.05 mM; pH 1

	Decolorization (%)								Mineralization (%)			
	[APS], mM				[HP], mM				[APS], mM		[HP], mM	
Time (min)	0.25	0.35	0.45	0.55	0.35	0.45	0.55	0.65	0.45	0.55	0.55	0.65
5	4.7	6.5	14.6	12.0	8.6	9.4	15.0	10.9	9.5	7.9	5.5	5.2
10	5.2	9.6	24.5	22.2	13.0	19.7	25.4	22.1	22.9	20.6	19.5	19.0
30	10.1	16.3	44.6	41.3	22.7	28.5	41.3	37.7	42.1	38.4	36.6	35.8
60	18.4	28.9	62.9	52.6	34.1	42.7	56.6	51.4	52.0	48.3	49.8	44.0
120	33.1	49.2	80.4	72.0	70.5	74.1	75.4	70.9	73.2	68.8	70.6	65.0
180	51.9	65.8	89.1	79.9	72.6	77.2	87.0	84.2	75.2	71.6	73.0	69.9
240	64.7	75.2	91.0	88.4	75.0	84.3	90.1	87.7	78.3	75.5	74.1	70.8

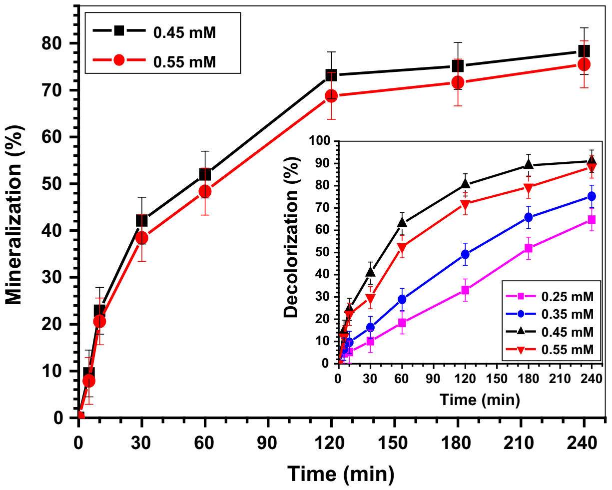

Fig. 3. Effect of APS concentration on the mineralization and decolorization (inset) of CCA: [CCA] = 0.05 mM; [Fe^{2+}] = 0.05 mM; pH 1.

$$SO_4^{\bullet-} + H_2O \rightarrow SO_4^{2-} + HO^{\bullet} + H^{+} \quad \text{(all pHs)} \tag{1}$$

$$SO_4^{\bullet-} + HO^{-} \rightarrow SO_4^{2-} + HO^{\bullet} \quad \text{(alikaline pH)} \tag{2}$$

3.2. Effect of metal ion concentration

The dependence of CCA decolorization and mineralization on the Fe^{2+} concentration in Fenton (Fe^{2+}/HP) process has been examined by performing experiments with different concentrations of Fe^{2+}. On increasing the concentration of Fe^{2+} progressively from 0.01 to 0.03 mM and then to 0.05 mM, the decolorization increases from 57.5 to 82.4% and then to 90.1% in 240 min of reaction (Table 2). It is noted that the reactions catalyzed by Fe^{2+} are nearly irreversible and, therefore, its role is to accelerate the generation of hydroxyl radicals. Thus, higher CCA decolorization at higher Fe^{2+} concentration may be considered due to the faster generation of $HO^{\bullet}$ and $SO_4^{\bullet-}$ radicals in the redox cycle of iron ($Fe^{2+} \leftrightarrows Fe^{3+}$) observed in Fenton process. In contrast to this expectation, the decolorization decreases from 90.1 to 86.9% when there is an increase in Fe^{2+} concentration from 0.05 to 0.07 mM (Fig. 4). This reduced catalytic activity of Fe^{2+} at higher concentration is further evidenced by the fact that there is a decrease in mineralization from 74.1 to 71.5% under similar conditions (Fig. 4). This indicates that excess of Fe^{2+} acts as a scavenger of $HO^{\bullet}$ radicals (Eq. (3)) [9,18]. Therefore, 0.05 mM of Fe^{2+} was considered as the optimum concentration in this experiment. This concentration of Fe^{2+} was also used with APS to find effect of equal concentration of Fe^{2+} on the optimum concentration of the oxidants, i.e. HP and APS. The other metal ion used for mineralization of CCA is Ag^{+} in the presence of HP and APS as oxidants. To evaluate the efficiency of equimolar concentration of both the metal ions, we have also considered 0.05 mM of Ag^{+} in the present study.

$$Fe^{2+} + HO^{\bullet} \rightarrow Fe-OH^{2+} \tag{3}$$

3.3. Effect of pH

The study on the effect of pH on the decolorization/mineralization process is essential as it is the pH of the solution, which primarily influences the generation of $HO^{\bullet}$ and $SO_4^{\bullet-}$ radicals. It may effect a change in species and/or precipitation of iron as ferric hydroxide, thereby reducing the efficiency of the Fenton process. It is a well-known fact that Fenton process is efficient at pH 2.5 to 3.0 and no such pH range has been recommended for $SO_4^{\bullet-}$-based oxidation process catalyzed by Fe^{2+} ion. In order to find the most suitable

Table 2
Effect of Fe^{2+} concentration on the decolorization and mineralization of CCA: [CCA] = 0.05 mM; [HP] = 0.55 mM; pH 1

	Decolorization (%)				Mineralization (%)	
	[Fe^{2+}], mM					
Time (min)	0.01	0.03	0.05	0.07	0.05	0.07
5	7.4	11.3	15.0	12.7	5.5	5.1
10	11.7	14.7	25.4	19.8	19.5	18.7
30	18.3	25.7	41.3	39.8	36.6	33.4
60	30.9	48.2	56.6	52.0	49.8	42.5
120	45.2	65.3	75.4	70.9	70.6	66.3
180	53.3	75.6	87.0	83.1	73.0	70.1
240	57.5	82.4	90.1	86.9	74.1	71.5

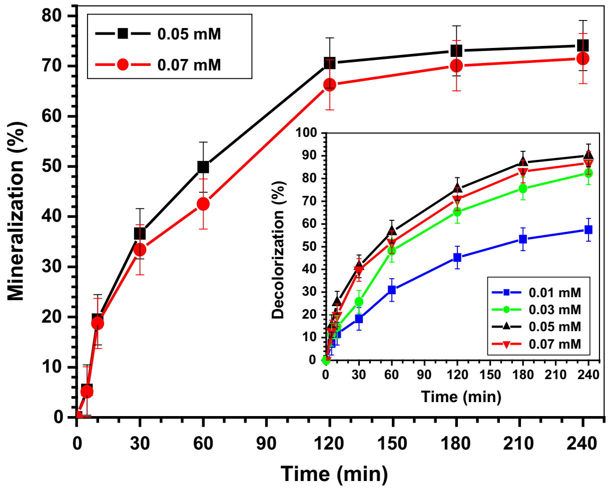

Fig. 4. Effect of Fe^{2+} concentration on the mineralization and decolorization (inset) of CCA: [CCA] = 0.05 mM; [HP] = 0.55 mM; pH 1.

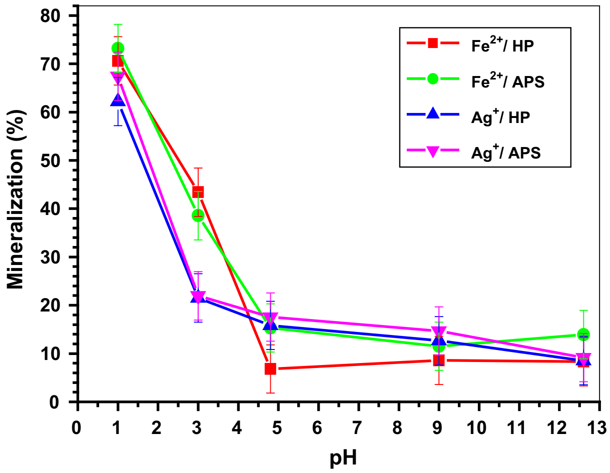

Fig. 5. Effect of pH on the mineralization of CCA by M^{n+}/oxidant systems: [CCA] = 0.05 mM; [HP] = 0.55 mM; [APS] = 0.45 mM; $[M^{n+}]$ = 0.05 mM; treatment period = 120 min.

pH for decolorization/mineralization of CCA, experiments with different systems (M^{n+}/oxidant) were performed in a pH range of 1–12.6. The variation of decolorization and mineralization with pH is presented in Table 3. The data show that highest decolorization (> 90%) is shown by Fe^{2+}/HP system at pH 3 and 12.6 and by Fe^{2+}/APS system at pH 3, 4.83, and 12.6; moderately higher by Ag^+-catalyzed systems at pH 4.83 (58.9% with HP and 51.9% with APS) and the least by Fe^{2+}/APS at pH 9 (35.3%). However, the mineralization pattern is interesting and provides unique result, in that all the systems yield maximum mineralization at pH 1 (Fig. 5) and their efficiency after a treatment period of 120 min follows the order: Fe^{2+}/APS > Fe^{2+}/HP > Ag^+/APS > Ag^+/HP. Thus, for a given metal, APS is found to be the preferred oxidant. Our effort to increase mineralization in Ag^+-catalyzed system by increasing treatment period to 240 min proved futile as there is only a marginal increase of 4% with either of the oxidants. Higher mineralization at pH 1 in M^{n+}/APS processes is obvious, which is due to the increased generation of $SO_4^{\cdot -}$ in acidic media (Eqs. (4) and (5)) [19]. It is expected that mineralization of CCA would proceed through the formation of HNO_3, H_2SO_4, and aliphatic acids such as muconic acid, maleic acid, fumaric acid, acetic acid, oxalic acid, and formic acid [16]. Thus, the pH of the solution after treatment is expected to decrease. Considering the millimolar concentration of the target molecule, the generation of the acids must be in the millimolar range. Since the initial pH of the solution is 1, no change in pH was observed after the treatment.

$$S_2O_8^{2-} + H^+ \rightarrow HS_2O_8^- \qquad (4)$$

$$HS_2O_8^- \rightarrow SO_4^{\cdot -} + SO_4^{2-} + H^+ \qquad (5)$$

Table 3
Effect of pH on decolorization and mineralization of CCA in different M^{n+}/oxidant systems: [CCA] = 0.05 mM; $[M^{n+}]$ = 0.05 mM; [HP] = 0.55 mM; [APS] = 0.45 mM; treatment period = 120 min

	Decolorization (%)				Mineralization (%)			
pH	Fe^{2+}/HP	Fe^{2+}/APS	Ag^+/HP	Ag^+/APS	Fe^{2+}/HP	Fe^{2+}/APS	Ag^+/HP	Ag^+/APS
1	75.4	80.4	74.1	74.9	70.6	73.2	62.2	67.4
3	93.3	96.1	71.4	67.8	43.4	38.6	21.5	22.0
4.83	87.9	95.4	58.9	51.9	6.8	15.3	15.8	17.6
9	86.8	35.3	69.4	66.8	8.6	11.5	12.7	14.7
12.6	99.2	90.3	72.2	69.6	8.3	13.9	8.6	9.2

The novelty of this study lies in the fact that the efficiency of Fenton process is maximum at pH 1 (Table 3) and probably we are reporting it for the first time. Thus, the earlier explanations for the reduced efficiency of Fenton process at pH 1 attributed to the scavenging of $HO^{\bullet}$ [20] as well as to the increased stability of HP as a result of formation of oxonium ion, $H_3O_2^+$ by the protonation of HP and thereby restricting the generation of $HO^{\bullet}$ [21] does not hold good in this case. This is quite interesting as Calcon, having similar structure as CCA (the only difference is the presence of additional –COOH group at C-3 position in CCA; inset of Fig. 1) under almost similar operational conditions, shows highest mineralization in Fenton process at pH 3 (35.6%) followed by at pH 1 (27.6%) [5]. Moreover, CCA shows significantly higher mineralization (70.6%) than Calcon at pH 1. On the other hand, both CCA and Calcon show highest mineralization at pH 1 in Fe^{2+}/APS system, the former showing 73.2% and the later 91.1% in 120 min. Thus, the introduction of a –COOH group in Calcon molecule at C-3 position has a marked effect on its mineralization process. While it increases from 27.6 to 70.6% on treatment with Fe^{2+}/HP by shifting the optimum pH from 3 to 1, it decreases from 91.1 to 73.2% with Fe^{2+}/APS at pH 1, i.e. without changing the optimum pH value. On increasing the pH to 3, mineralization of CCA by both the processes is reduced considerably. Nevertheless, Fe^{2+}/HP and Fe^{2+}/APS systems show moderately higher mineralization than Ag^+-catalyzed systems. Further, Fe^{2+}/HP system shows higher efficiency at pH 3 than Fe^{2+}/APS system with the former showing 43.4% and the later 38.6% mineralization (Table 3). On further increasing the pH, the mineralization in all the systems is considerably lowered, even falls below their practical applicability. The remarkable lowered mineralization at pH ≥ 5 may be due to the formation of $Fe(OH)_3$ which promotes the decomposition of HP into O_2 and H_2O [22] as well as due to the reduced oxidation potential of HP at higher pH and oxidation of NaOH by HP [2]. Thus, Fe^{2+}/APS system at pH 1 is most suitable for treating wastewater containing CCA. The UV–vis absorption spectra of aqueous solution of CCA at different intervals of treatment with the most effective system under optimized conditions are presented in Fig. 6. The absorption of peak at 236 nm, attributed to $\pi \rightarrow \pi^*$ transition within the benzene ring, shows an increment with time. This increment may be due to the generation of acids like maleic acid, acrylic acid, and methyl acrylic acid, which exhibit characteristic absorption in this region [23]. On the contrary, the absorbance of the other peak at 530 nm, attributed due to $n \rightarrow \pi^*$ transitions of N=N, C=N, and C=O chromophore groups, diminishes with time and reaches 80.4% in 120 min. This indicates the breaking of N=N and/or C–N bonds in CCA molecule.

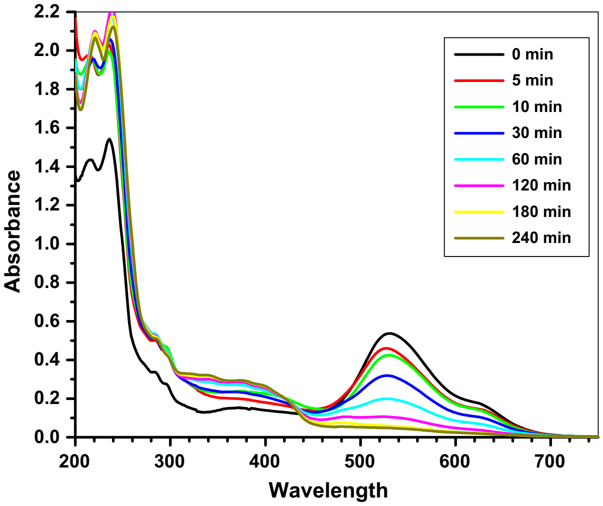

Fig. 6. UV–vis absorption spectra of aqueous solution of CCA in Fe^{2+}/APS system: [CCA] = 0.05 mM; [APS] = 0.45 mM; $[Fe^{2+}]$ = 0.05 mM; pH 1.

3.4. Bioassay of post-treated solutions

In the previous section, it has been shown that both Fenton and Fenton-like processes show highest mineralization at pH 1. Further, Fe^{2+}-catalyzed systems are found to be more efficient towards mineralization than Ag^+-catalyzed systems. In order to evaluate the practical applicability of these processes, the relative biotoxicity of the treated solutions was assessed by growing *E. coli* in the solutions after adjusting to various pH values. The relative biotoxicity of the treated solutions presented in the study was with respect to the control as well as the pure dye. It is noted that the solutions are acute toxic at pH values 1 and 2 as is evidenced by the fact that no *E. coli* stain is observed at these pH values. On increasing the solution pH, the biotoxicity is reduced and becomes least at pH 7, beyond which the biotoxicity increases further and becomes acute toxic again at pH 11 and 12 (Fig. 7). It is important to note that biotoxicity is least at pH 7 in all the systems. Nevertheless, the solutions are still nearly 50% more biotoxic than the untreated dye in all systems except in Ag^+/HP, where it is 56%. A similar result was reported by us recently on the treatment of Calcon by Fenton and Fenton-like processes using different metal ion catalysts, and the biotoxicity in the treated solutions has been described to be due to the presence of metal ions in the treated solutions [5]. The effect of presence of –COOH group

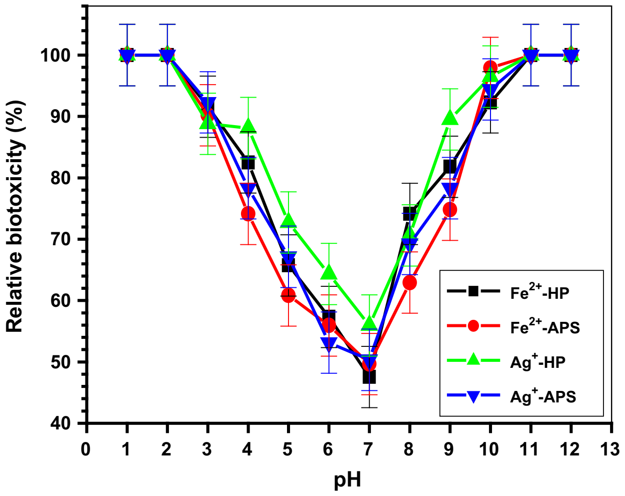

Fig. 7. Effect of pH on the toxicity of the treated CCA solutions: [CCA] = 0.05 mM; [M^{n+}] = 0.05 mM; [HP] = 0.55 mM; [APS] = 0.45 mM; pH of treatment = 1; treatment period = 120 min; Inoculum (*E. coli*) = 100 μl. Relative biotoxicity indicates biotoxicity with respect to the original dye solution.

at C-3 position is clearly observed in the biotoxicity study of treated solutions. On removing the –COOH group, a sharp decrease in biotoxicity from 50 to 22.7% and 26.7% is observed in Fe^{2+}/APS and Ag^{+}/APS systems, respectively [5].

4. Conclusion

The present study aims at selecting most suitable operational parameters to achieve maximum mineralization by Fenton and Fenton-like processes using Fe^{2+} and Ag^{+} as metal ion catalysts and HP and APS as oxidants. It also aims at generating post-treated solutions devoid of biotoxicity. The results show the most suitable process for mineralization of CCA is Fe^{2+}/APS at pH 1. One important finding of this study is that Fenton process shows higher efficiency at pH 1 than at pH 3. The presence of a –COOH group in CCA at C-3 position has a marked effect on its mineralization process. It causes a shift in the optimum pH value of Fenton process from 3 to 1. It is noted that removal of –COOH group has opposite effect on mineralization by Fe^{2+}/HP and Fe^{2+}/APS processes at pH values 1 and 3. While in the former, it is reduced from 70.6 to 27.6% at pH 1 and from 43.4 to 35.6% at pH 3, in the latter it is increased from 73.2 to 91.1% at pH 1 and from 38.6 to 42% at pH 3. In other words, mineralization is favored by Fe^{2+}/HP process in the presence of –COOH group and by Fe^{2+}/APS process in its absence. Bioassay experiments on treated solutions reveal that they are ≥50% biotoxic than the untreated dye in 120 min of treatment. The biotoxicity can be reduced to 22.7 and 26.7% in Fe^{2+}/APS and Ag^{+}/APS systems, respectively, when the –COOH group is removed. Thus, metal ion-catalyzed treatment of CCA in the presence of oxidants is not environmentally benign under the present experimental conditions.

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