Desalination and Water Treatment
www.deswater.com

doi:10.5004/dwt.2017.21000

82 (2017) 355–361
July

Evaluation of chemical, biological and ecotoxicological characteristics of cresol and its Fenton's degradation products

Mahmood Hajian[a,*], Seyed Ahmad Mirbagheri[a], Iman Taqizade[b], Amir Hosein Javid[a]

[a]*Department ofEnvironmental Engineering, Faculty of Environmental and Energy, Science and Research Branch, Islamic Azad University, Tehran, Iran, Tel./Fax +982188770006, email: mhajian44@yahoo.com (M. Hajian), seyedahmad_mirbagheri@yahoo.com (S.A. Mirbagheri), ahjavid@gmail.com (A.H. Javid)*
[b]*Department of Chemistry, Faculty of Science, Tehran University, Tehran, Iran, email: i.taqizade@yahoo.com*

Received 4 December 2016; Accepted 29 May 2017

ABSTRACT

In this study, chemical (COD, TOC and MOSC), biological (BOD) and ecotoxicological (GI, EC50 and TU) characteristics of cresol and its Fenton's degradation products were evaluated comparatively. Experiments were carried out in previously optimized conditions. The degradation efficiency and mineralization efficiency of the process was 82% and 41.7% respectively. MOSC changed from −0.875 to +2.499. According to HPLC analysis results after the Fenton process, cresol was completely removed. The Germination Index (GI) improved from 33.1% to 59.7%. Based on TU results, cresol phytotoxicity is 3.5 times greater than its final degradation products. Despite these results, the ecotoxicity and biodegradability of intermediates and products were very unsuitable for the discharging of treated wastewater to the environment. Lime post-treatment was used as an efficient detoxification of cresol final degradation products.

Keywords: Cresol; Fenton's degradation products; Germination index; COD; Detoxification

1. Introduction

Cresol, methylphenol, occurs in three isomeric forms: o-cresol (2-methylphenol), m-cresol (3-methylphenol) and p-cresol (4-methylphenol).The mixture of o-, m-, and p-cresols is referred to in the technical literature as tri-cresol. Cresols are used as a starting material for the synthesis of herbicides, insecticides, resins, antioxidants and pharmaceuticals [1]. The major sources of cresols in the environment are wastewater from coal gasification, fractional distillation of coal tars, petroleum refineries and phenolic resin industries [2–4]. Cresols are persistent compounds and are toxic to a wide spectrum of organisms. They may have adverse effects on ecosystems and human health [5]. In 1979, cresols were classified by the Environmental Protection Agency (EPA) as persistent, toxic and a priority [6]. In recent years, the EPA has classified cresols within group C: possible human carcinogens [7]. Thus, they constitute a threat when released into the environment [8].

The removal of cresols may be accomplished through physical, biological and chemical processes. Adsorption on an adsorbent is one of the physical methods. The adsorption of cresol on PAC (powdered activated carbon) [9] and on carbonylated hypercrosslinked polymeric adsorbent [10] was investigated. Several microorganisms utilize cresol as a sole carbon source, in spite of its toxicity [11]. Although cresols can be degraded by Fungi (*Mortierella sarnyensis* [8]), the most studied microorganisms are *Pseudomonas* bacteria. Kaymaz studied the biodegradation of o-cresol by *Pseudomonas putida* DSM 548 [12] and Aneez Ahamad investigated the biodegradation of cresols by *Pseudomonas sp. CP*$_4$ [13]. The chemical treatment methods included conventional and advanced oxidation processes (AOPs). In the conventional oxidation processes, common oxidizing agents such as ozone are used for cresol degradation [14]. AOPs consist of near ambient temperature and pressure water treatment processes which involve the generation of hydroxyl radicals (HO•) in sufficient quantity to affect water purification

*Corresponding author.

[15]. Among AOPs, the Fenton process ranks as a widely studied and applied catalytic method, based on the generation of hydroxyl radicals from hydrogen peroxide with ferrous (Fe^{2+}) ions acting as homogeneous catalysts at acidic pH and ambient conditions [16].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\circ} + OH^{-} \quad (1)$$

Kavitha and Palanivelu investigated cresol degradation by the Fenton process [17]. They observed that the degradation efficiency for cresol isomers was 82% within 120 min at optimum conditions. They also studied COD and TOC removal.

In this study, different chemical, biological and ecotoxicological characteristics of cresol and its degradation products during the Fenton process were investigated comparatively and the suitability of the Fenton process for treatment and detoxification of cresol was evaluated.

2. Materials and methods

2.1. Materials

Tri-Cresol (mixture of isomers) were prepared using equal amounts of o-cresol (99%; for synthesis), m-cresol (99%; for synthesis) and p-cresol (98%; for synthesis). Technical grade hydrogen peroxide (35%), analytical grade sodium hydroxide (99%), iron (II) sulfate heptahydrate (99.5%), sulfuric acid (97%) and calcium hydroxide (96%) were used. All reagents were supplied by MERCK KGaA (Darmstadt, Germany). For pH adjustments, sulfuric acid and sodium hydroxide 20% solutions were used. Phytotoxicity tests were carried out with garden cress seeds (*Lepidium sativum L.*) from a local source.

2.2. Experimental procedure

The degradation of cresol was performed in a similar procedure as described in [17] (the conventional Fenton process). After dissolving 200 mg of tri-cresol and 0.22 g of iron (II) sulfate heptahydrate in distilled water in a beaker, the pH of this solution was set at 3.0 by adding diluted sulfuric acid (20%). The reaction was initiated after adding 3.21 g hydrogen peroxide (33.4%; freshly titrated) into the beaker at room temperature. During the reaction, the solution was mixed by a mechanical stirrer (500 rpm).

2.3. Analysis

The initial pH of the solution was measured using *WTW pH meter inoLab 720* (Germany). Samples were withdrawn at predefined time intervals from the reactor (at 5, 10, 15, 20, 30, 45, 60, 90 and 120 min from the start). All of the samples were immediately quenched by adding sodium hydroxide solution (20%) to raise their pH to 12. The COD (chemical oxygen demand) and BOD (biological oxygen demand) of the samples were measured according to standard methods of APHA [18]. The TOC (total organic carbon) was measured using *Shimadzu TOC analyzer TOC5000* (Germany). Specific quantitative analysis of cresol was carried out in High Performance Liquid Chromatography using a Knauer K2500 HPLC, with a C18 (250 × 4.6 mm) column and NB166UV detector. Mixtures of water/methanol/acetic acid (59.4/39.6/1 v/v) were used as mobile phase at a flow rate of 0.8 mL/min. The quantitative determination of acetic acid and oxalicacid were carried out in *MetrohmIon Chromatography861 Advanced Compact* (Switzerland) with a *Metrosep A Supp 5* (150 mm long) as analytical column. The isocratic mobile phase consisted of 4.5 mM Na_2CO_3 and 1.5 mM $NaHCO_3$, operated at a flow rate of 0.8 mL/min. The peaks were detected at 4.00 and 11.86 min retention time for acetate and oxalate ions respectively.

Phytotoxicity tests were conducted in order to evaluate the ecotoxicity of cresol and its degradation products using an indirect acute toxicity bioassay on the dicotyledonous plant *Lepidium sativum L.* according to a standardized protocol [19]: three days of seed germination and root growth tests were conducted using the dicotyledonous garden cress *Lepidium sativum L.* as indicator. The toxicity tests were conducted using 20 seeds each for germination on Petri dishes. 3mL of solutions were used to wet the paper supporting the seeds. The Petri dishes were kept at room temperature (~25°C) for 72 h. The number of seeds germinated, formed biomass, stem length and root length were measured to determine the relative bioassay and germination index (GI).

3. Results and discussion

3.1. Degradation efficiency

The overall degradation of cresol was monitored by COD measurements and degradation efficiency was calculated as follows:

$$Degradation\ efficiency = \frac{COD_{initial} - COD_{sample}}{COD_{initial}} \quad (2)$$

Table 1 presents the results of the COD measurements and Fig. 1 shows the results of degradation efficiency and the degradation rate of cresol in each sample.

3.2. Mineralization efficiency

The mineralization of cresol was monitored by TOC measurements and mineralization efficiency was calculated as follows:

$$Mineralization\ efficiency = \frac{TOC_{initial} - TOC_{sample}}{TOC_{initial}} \quad (3)$$

Table 1 presents the results of the TOC measurements and Fig. 2 shows the results of mineralization efficiency and mineralization rate of cresol in each sample.

The Initial MOSC (–0.875) was in accordance with calculated MOSC of cresol (–0.856).

3.3. Oxidation efficiency

The average Oxidation state of organic compounds in treated solution was estimated by MOSC (mean oxidation state of carbon) calculations as follows [20]:

Table 1
Results of COD, TOC and MOSC analysis of samples

	Time (min)								
	0	5	10	15	30	45	60	90	120
COD (mg O_2/L)	507	445	396	352	248	174	123	99	91
TOC (mg /L)	156	143	133	124	111	104	99.5	91.4	90.9
MOSC	−0.875	−0.654	−0.476	−0.258	0.654	1.478	2.149	2.376	2.499

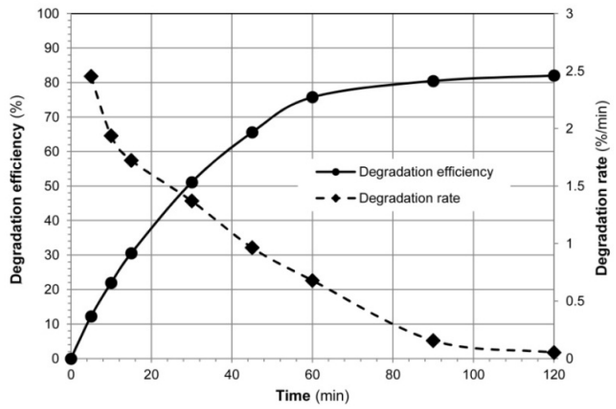

Fig. 1. Degradation efficiency and degradation rate for cresol during Fenton process.

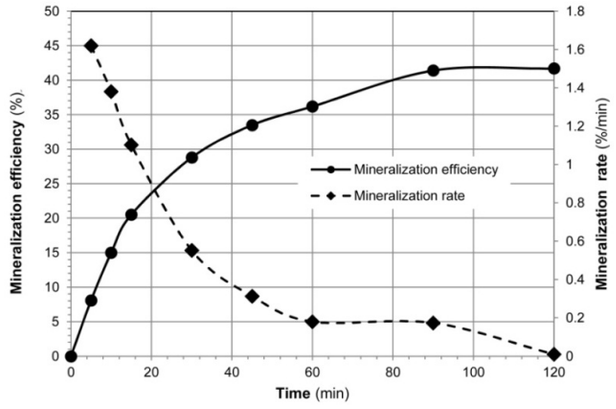

Fig. 2. Mineralization efficiency and mineralization rate for cresol during Fenton process.

$$MOSC = 4 - 1.5\frac{COD}{TOC} \tag{4}$$

Table 1 presents the results of the MOSC and Fig. 3 shows the result of MOSC and oxidation rate in each sample.

3.4. Cresol degradation

The specific degradation of cresol was monitored by the HPLC method for initial and final samples (time of sampling = 0 and 120 min). In the initial sample, cresol was detected in chromatogram as main peak (retention time = 21.8 min). In the final sample no evidence of cresol was detected (<0.1 ppm) and only one peak in retention time of 6.96 min was observed. This peak belonged to oxalic acid. Carboxylic acids could not be quantitatively analyzed by the HPLC method and the amount was measured by Ion Chromatography (section 3.6.)

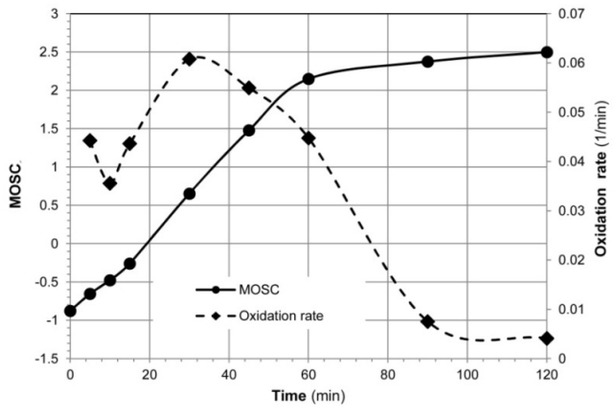

Fig. 3. MOSC and Oxidation rate for cresol during Fenton process.

3.5. Biological oxygen demand tests

Table 2 presents the results of the BOD measurements in four samples withdrawn during Fenton process.

3.6. Ecotoxicity tests

The phytotoxicity of cresol and its degradation products was assessed by germination of *Lepidium sativum L* (Fig. 4). All samples were neutralized (pH = 7.0) before performing toxicity tests.

Relative toxicities were calculated as follows:

$$RL_S = \frac{L_{S\ blank} - L_{S\ sample}}{L_{S\ blank}} 100 \quad \left(L_S = Length\ of\ stem\right) \tag{5}$$

$$RL_R = \frac{L_{R\ blank} - L_{R\ sample}}{L_{R\ blank}} 100 \left(L_R = Length\ of\ root\right) \tag{6}$$

$$RM = \frac{M_{blank} - M_{sample}}{M_{blank}} 100 \left(M = biomass\right) \tag{7}$$

In all germination tests, the percentages of relative seed germination (RSG), relative root growth (RRG) and germination index (GI) were calculated as follows [21]:

$$RSG = \frac{number\ of\ seeds\ germinated\ in\ sample}{number\ of\ seeds\ germinated\ in\ blank} 100 \quad (8)$$

Table 2
Biological Oxygen demand during Fenton process

	Time (min)			
	0	10	45	120
BOD (mg/lit)	91	29	20	20
BOD/COD	0.1795	0.0732	0.1149	0.2198

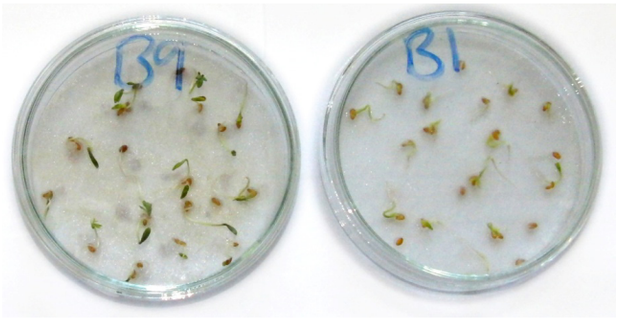

Fig. 4. *Lepidium sativum L.* germination in the presence of cresol (right) and its degradation products (left).

$$RRG = \frac{mean\ root\ length\ in\ sample}{mean\ root\ length\ in\ blank} 100 \quad (9)$$

$$GI = \frac{RSGRRG}{100} \quad (10)$$

Table 3 presents the results of the germination of seeds in the samples. Fig. 5 shows the results of phytotoxicity tests and Fig. 6 shows the GI in each sample.

For a more detailed investigation of phytotoxicity, EC50 of cresol and its final products in the Fenton process were determined. Toxicity data is usually expressed as a concen-

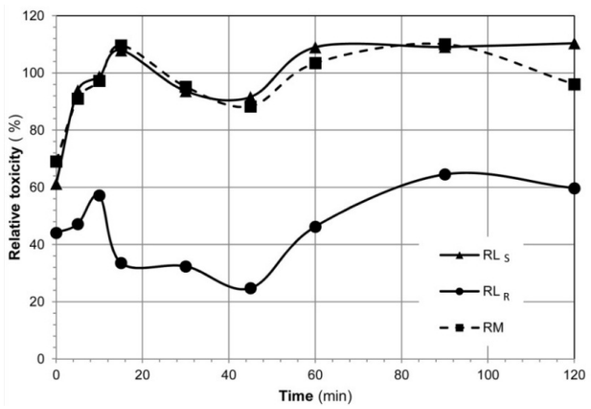

Fig. 5. Relative bioassay for cresol and its degradation products during Fenton process.(RM: relative biomass, RL_S: relative stem length, RL_R: relative root length).

Table 3
Results of phytotoxicity evaluation of samples

	Time (min)									Blank
	0	5	10	15	30	45	60	90	120	
L_S Stem length (mm)	12.1	18.5	19.5	21.3	18.5	18.1	21.5	21.5	21.8	19.7
RL_S Relative stem length (%)	61.3	93.9	98.7	108	93.7	91.6	109	109	110	100
L_R Root length (mm)	9.53	10.2	12.4	7.26	7	5.35	10	13.9	12.9	21.6
RL_R Relative root length (%)	44.1	47.2	57.2	33.6	32.4	24.8	46.3	64.6	59.7	100
M Biomass (mg)	8.27	10.9	11.7	13.1	11.4	10.6	12.4	13.2	11.5	12
RM Relative biomass (%)	69.1	91	97.3	110	95.2	88.4	103	110	96.1	100
RSG Relative seed germination (%)	75	100	100	95	100	100	85	90	100	100
RRG Relative root growth (%)	44.1	47.2	57.2	33.6	32.4	24.8	46.3	64.6	59.7	100
GI Germination index (%)	33.1	47.2	57.2	31.9	32.4	24.8	39.4	58.1	59.7	100

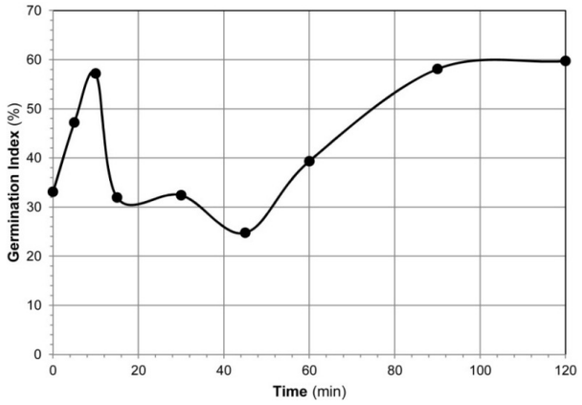

Fig. 6. Germination index (GI) for cresol and its degradation products during Fenton process.

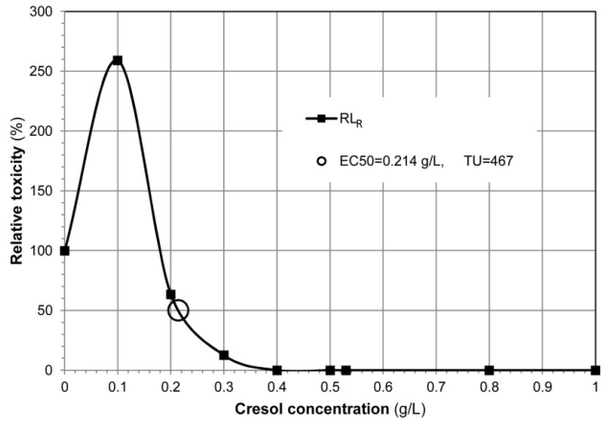

Fig. 7. EC50 for cresol on root growth of *Lepidium sativum L.*

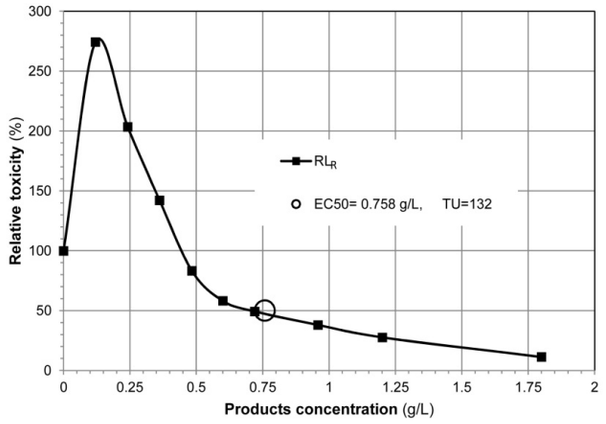

Fig. 8. EC50 for cresol final degradation products (oxalate + acetate) on root growth of *Lepidium sativum L.*

tration causing a specific effect (e.g. death or growth) in 50% of the tested organisms (effect concentration, EC50). As the most pronounced effect of cresol and its products is on root length, in this study EC50 stands for the concentration of a test compound that reduces root growth to 50% of control [22,23].

Kavitha [17] reported that the final product of cresol degradation in the Fenton process is a mixture of oxalic and acetic acid. According to his results, 92% of organic products of cresol degradation are these acids. According to our ion chromatography results, oxalic and acetic acid were produced in approximately 110:140 weight ratios. Therefore a series of cresol solutions in distilled water with different concentrations and a series of neutralized oxalic and acetic acid solutions (pH = 7.0) in distilled water with different concentrations and a fixed ratio (110:140) were prepared for the toxicity tests. Figs. 7 and 8 show the results of toxicity evaluation for cresol and its synthetic final products respectively. The experimental data was analyzed by regression analysis. According to these results, EC50 for cresol and synthetic final products is 0.214 g/L and 0.758 g/L respectively.

For a better interpretation of toxicity data, measurements were converted into Toxicity Units (TU), i.e. the inverse of EC50 values, expressed in percent [24]. According to the toxicity classification system (TCS) reported by [25], we can consider four classes of ecotoxicity: class 1 (TU < 1) exhibiting no significant ecotoxicity, class 2 (1 < TU < 10) exhibiting significant ecotoxicity, class 3 (10 < TU < 100) exhibiting high acute ecotoxicity, and class 4 (TU > 100) exhibiting very high ecotoxicity. Following this classification, cresol (TU = 467) and its final degradation products (TU = 132) would be characterized as displaying very high ecotoxicity (class 4).

3.7. Detoxification

As mentioned above, oxalic acid is one of the cresol final degradation products in the Fenton process and the conventional Fenton process cannot degrade it [17]. According to very low calcium oxalate solubility in water (0.61 mg/L at 20°C [26]), oxalic acid can be removed from wastewater in the form of calcium oxalate. Therefore, the last sample of the Fenton process (time of sampling: 120 min) was treated with lime ($Ca(OH)_2$) in 200% excess of Ca^{2+}. After filtration and pH neutralization, oxalate content of the sample was negligible and COD of sample decreased from 91 to 40 mg O_2/L. Phytotoxicity of this sample was also evaluated. Table 4 presents the results of the germination of *Lepidium sativum L.* seeds in this sample.

Table 4
Effect of lime post-treatment on phytotoxicity of cresol degradation final products

	GI (%)	RL_R (%)	RL_S (%)	RM (%)
Before lime treatment	59.7	59.7	110	96.1
After lime treatment	158	158	144	124

According to these results, Ca^{2+} has a strong detoxification effect on cresol final degradation products in the Fenton process. The results that are greater than 100% are most likely due to the fertilization effect of remained acetate.

4. Conclusions

According to this study, the most degradation and mineralization of cresol took place during the first hour of the process and after two hours this process was stopped. The maximum degradation and mineralization efficiencies for cresol were 82% and 41.7% respectively. The maximum oxidation rate of cresol was observed at 30 min after the start. MOSC of solution was varied from –0.875 to 2.499.

According to chromatographic results (HPLC and IC) after the Fenton process no cresol remained in the final sample (<0.1 ppm) and the main products of the process were acetic and oxalic acids.

BOD of solution varied from 91 to 20 during the Fenton process. However, BOD/COD ratio improved from 0.1795 to 0.2198 – nonetheless this ratio is too low for biological post-treatment. Biological methods can effectively treat wastewaters with high biodegradability ratio (BOD/COD > 0.4) [27].

Unlike COD, TOC and BOD trends, the trend of GI was non uniform. At the beginning of the reaction, GI had an increasing trend then decreasing trend and again increasing trend. The most toxicity of solution was observed in 45 min after start. This phenomenon is probably due to high toxic intermediate. The GI of solution ultimately improved from 33.1% to 59.7%.This value is too low for allowable discharging of treated wastewater to the environment. For comparison, it has been noted that a GI value above 80% indicated the disappearance of phytotoxins in composts [28,29].

The phytotoxicity of cresol and its final degradation product (mixture of oxalate and acetate) were 467 and 132 respectively in terms of TU. Although the phytotoxicity of the final degradation product is one third of cresol, both of them would be characterized as class 4 – exhibiting very high ecotoxicity. This phytotoxicity of final degradation products can be reduced by lime treatment.

In conclusion, despite the Fenton process' suitability as a treatment for degradation of cresol, according to ecotoxicity and biodegradability tests it is an unsuitable process for discharging treated wastewater to environment and if it runs in wrong way can expose highly toxic intermediates to the environment. Lime post-treatment can be used as an efficient detoxification of cresol final degradation products (i.e. oxalate). This post-treatment also improves COD removal from 82% to 92%.

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