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Toxicity evaluation of phenol by-products resulted from degradation of phenol by Fe (III)-doped TiO_2/UV process

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ABSTRACT

The objective of this study was the evaluation of phenol toxicity and by-products resulted from its degradation by Fe (III)-doped TiO_2/UV process. 10 mg/L of phenol solution was manipulated for degradation by Fe (III)-doped TiO_2 (synthesized by sol-gel method) under UV ray. By-products detected using GC-MS. In order to toxicity assessment of phenol by-products, the effluent was used in bioassay tests performed using *Daphnia magna* at 24, 48, 72, and 96 h exposure and 50% lethal concentration (LC_{50}) was determined using probit analysis in SPSS ver. 16.0 software. Results indicated that after 210 min, phenol concentration was decreased to 1.022 mg/L. Detected by-products consisted of catechol, resorcinol, hydroquinone, glycerol, glutaric acid, oxalic acid, 1,2,3–benzenetriol, phenol, acetic acid, and E–2–butenedioicacid. According to the obtained values of LC_{50}s, the effluent toxicity was 6–9-fold higher than that of initial phenol solution at all of the exposure times. Results of the present study confirmed that the by-products of phenol oxidation by Fe(III)-doped TiO_2/UV may introduce more toxic effects on *Daphnia magna* and this phenomenon should be considered in the future applications.

Keywords: Phenol; By-product toxicity; Fe (III)-doped TiO_2; *Daphnia magna;* LC50

1. Introduction

The increasing environmental toxic wastes leads to progressive retrogression in our quality of life. These conditions cause the world's scientific society efforts to search for efficient means of environmental remediation with the aim of human health and nature protection [1]. Phenol and phenolic compounds are potentially toxic for the environmental and human health [2]. These compounds are a main group of industrial effluent pollutants produced by chemical, petrochemical, pharmaceutical, paint, coal carbonization, tar distillation units, coke ovens, textile, pesticide plants, food and beverage, the production and recycling of rubber goods and biotechnological industries, bisphenol, and other synthetic resin manufacturing units industries [1,3]. According to the previous investigations, phenol and its derivatives are found in the environmental samples both as natural and artificial mono-aromatic products, and that they are among the most common water, wastewater, and air pollutants [1,4].

Phenol leads to toxic effect and adds odor to water even in trace quantities [5]. Also, inhibition of biological activities in wastewater treatment plants is reported in the pres-

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ence of this compound. Hence, phenolic compounds can strongly reduce the biodegradation of other components [6,7]. Phenols negative effects on human health, environment and life quality have been described in previous literatures [1,8].

In previous decades, depending on quality and quantity of wastewaters, some of the conventional methods such as solvent extraction, incineration, chemical oxidation, free-cultured biological degradation, granular activated carbon (GAC) adsorption, and membrane separation have been applied for treatment of phenol and phenolic compounds [3]. Nowadays advanced techniques such as photo-assisted catalytic decay are commonly applied for degradation of phenolic compounds [7]. Researches show that titanium dioxide (TiO_2) regarding to its biological and chemical inertness, non–toxicity, strong oxidizing power, insolubility, relatively low cost and long-term stability against chemical and photo-corrosion, is the most suitable catalyst for environmental applications among different oxide semiconductor photocatalysts [9–11]. Some researchers have indicated that photocatalytical efficiency of TiO_2 increases when it is doped with transition metal cations such as Pt, Au, Pd, Ru, and Fe because of reducing electron–hole recombination rate [9–13]. This has been showed that Fe (III) is a successful doping material among other metallic ions [9,12,13].

Other investigations have shown that Fe(III)-doped TiO_2 nanoparticles synthesized by sol-gel method have lower toxic for *Daphnia magna* in comparison with TiO_2 nanoparticles [14].

Phenol can be completely degraded by Fe (III)-doped TiO_2/UV process [7] but the previous study showed that final products may consist of toxic compounds. In fact this process can produce some by-products that may be more toxic than the initial influent compounds [7].

The objective of this study was toxicity evaluation of phenol by-products resulted from its degradation by Fe (III)-doped TiO_2/UV process. For this purpose phenol solution was manipulated for degradation by Fe (III)-doped TiO_2 (synthesized by sol-gel method) under UV ray. Then for toxicity assessment of phenol by-products, the effluent of this process was used in toxicity tests. Bioassay tests were performed using *Daphnia magna*due to its high sensitivity to the phenol [15], and also their large size that makes them easy to use [16].

2. Material and methods

2.1. Synthesis of Fe (III)-doped TiO_2 by sol-gel method

Fe (III)-doped TiO_2 was prepared according to Fig. 1 a clear account on the method and reagents used are given by previous studies [17].

2.2. Phenol degradation and by-product detection

Photocatalytic degradation of phenol was investigated with 10 mg/L of initial phenol concentration and at optimal conditions [7] (pH = 3 and Fe(III)-doped TiO_2 = 0.5 g/L) under UV irradiation (A 27 W low pressure lamp). pH was adjusted by H_2SO_4 (0.1 mol/L). The suspension was stirred in the dark for 30 min to achieve adsorption/desorption equilibrium before irradiation. After irradiation time (210 min), for separation of Fe (III)-doped TiO_2 particles, centrifuging the solution at 6000 r/min for 10 min and filtration were performed subsequently. The UV–vis spectrophotometer (Perkin–Elmer Lambda 25) was used for determination of phenol concentration and was set at a wavelength of 500 nm [16]. For determination of phenol and by-products of its photocatalytic degradation under Fe (III)-doped TiO_2/UV process, a GC-Ms system (Agilent Technologies, Palo Alto, CA, USA), equipped with a 5973 mass selective detector quadrupole was used. The gas chromatograph was fitted with a DB5 capillary column (30 m, 0.25 mm id, 0.25 µm film thickness). The instrumental temperature was as

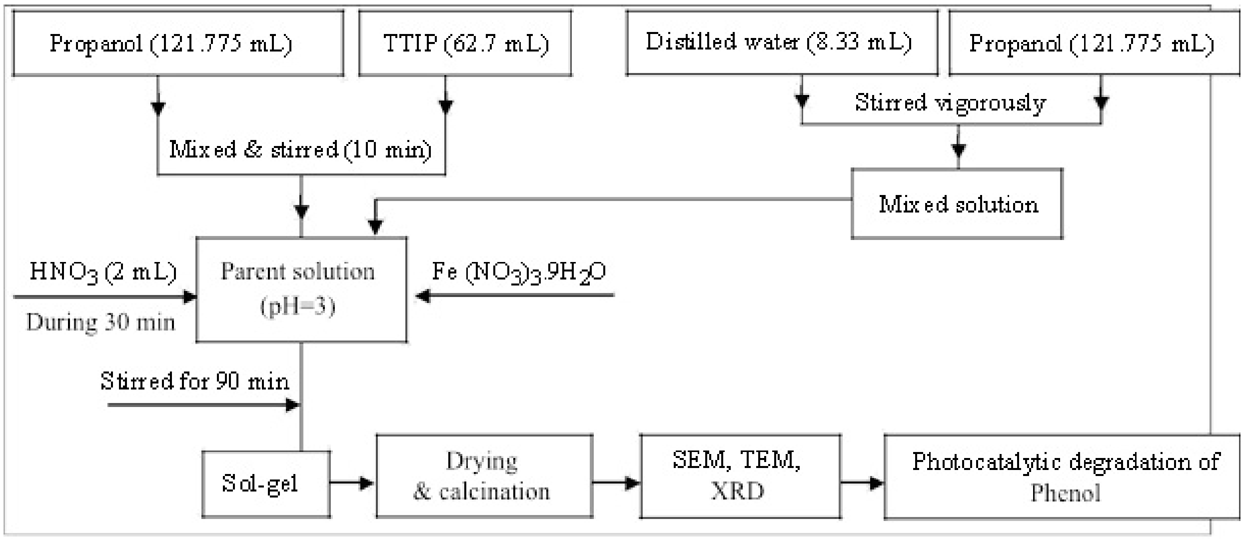

Fig. 1. A brief scheme of synthesis stages of Fe (III)-doped TiO_2 by sol-gel method for applying in the photocatalytic degradation. of phenol (TTIP: Titanium tetraisopropoxide; SEM: Scanning electron microscopy; TEM: Transmission electron microscopy; XRD: X-ray diffraction).

following: injector temperature 200°C; initial oven temperature 30°C (held for 2 min), increased to 280°C at a rate of 10°C/min, and held for 1 min. The inlet sample was operated in splitless mode. The temperature of the transfer line was maintained at 210°C. Helium (99.999%) was used as the carrier gas at 1.5 mL/min. The source and quadrupole temperatures were kept at 230°C and 150°C, respectively. The electronic beam energy of the mass spectrometer was set at 70 eV. The mass selective detector was operated in scan mode.

2.3. Culturing of *Daphnia magna*

Only 20–30 organisms are needed to start culturing of *Daphnia magna* [16]. These organisms were provided from the Environment Faculty of Tehran University (Tehran-Iran). According to the procedure described in standard No. 8711 of standard methods for the examination of water and wastewater [16], a 3.8 L glass vessel containing 3 L culture medium was used for mass production and 30 mg yeast powder was added every other day. Water hardness was adjusted to 160–180 mg $CaCO_3$/L by dissolving salts in distilled or deionized water [16]. Although *Daphnia* lives in a wide range of pH, but pH was maintained at optimal range of 7–8.6. To ensure a supply of oxygen for aerobic growth, aeration was performed weekly using air aeration pump.

To initiate toxicity test, *Daphnia magna* neonates (≤ 24 h old) was used [16,18]. To obtain neonates for a test, females bearing embryos were eliminated from the stock culture 24 h before starting the test and were put in 400-mL vessel containing 300 mL of the medium [16]. It is feasible to get these neonates through 5 vessels each containing 10 mature *Daphnia* [19].

2.4. Toxicity measurement of phenol and its by-products to *Daphnia magna*

At this stage, 20, 25, 50, 60, 80 and 100% (V/V_0) of both initial phenol solution (C_o = 10 mg/l) and effluent after Fe(III)-doped TiO_2/UV treatment (t = 210 min) were diluted with dilution water and 6 samples for them were prepared (at least 5 concentrations of experimental sample are necessary for determination of LC_{50} value) [16]. Then, the infants of *Daphnia magna* which were released from mother bags during the last 24 h at 20–25°C, were inseminated to experimental samples (10 infants to each sample) [16]. A 10 mL glass pipette was used for gathering and transmission of *Daphnia*. For determination of acute toxicity by *Daphnia*, 48 h exposure time is considered to be acceptable [20]. But in this research in order to investigate the effect of contact time, 24, 48 h, 72 h and 96 h were considered. Then, the immobile *Daphnia* in each sample were counted and LC_{50} values were calculated using the probit analysis. The obtained data from probit analysis were used in Excel software to plot graphs. The toxicity values were converted to toxic units (TU) according to: TU = 100%/LC_{50}. [15,21].

3. Results and discussion

The x-ray diffraction (XRD) spectrum of the sample showed the presence of both rutile and $bTiO_2$ or nanorod TiO_2 phases (Fig. 2). No sign of iron containing phases was distinguished in these diffractograms, which that indicated the amounts of Fe were lower than the values that could be detected by XRD. The scanning electron microscopy (SEM) images of Fe (III)-doped TiO_2 nanoparticles proved the presence of $bTiO_2$. The particle size distribution determined by SEM images was less than 50 nm (Fig. 3). The atomic ratio of Fe to Ti was 0.034%, which estimated using the energy dispersive x-ray (EDX) analysis.

Fig. 4 shows the degradation rate of phenol under exposure to Fe (III)-doped TiO_2/UV at 210 min. Results indicated that phenol concentration has been decreased to 1.02 mg/L after photocatalytic treatment during 210 min (degradation degree about 90%).After this degradation, the effluent was analyzed by GC–MS and the by-products were identified by the use of instrument library (Fig. 5). These by-products consist of catechol, resorcinol, hydroquinone, glycerol, glutaric acid, oxalic acid, 1,2,3–benzenetriol, phenol, acetic acid, and E–2–butenedioic acid. The small peaks in the Fig. 5 could not be analyzed because the MS information was not enough.

Guo et al. [22] have reported that the mechanism of phenol photodegradation by catalysts such as TiO_2 includes

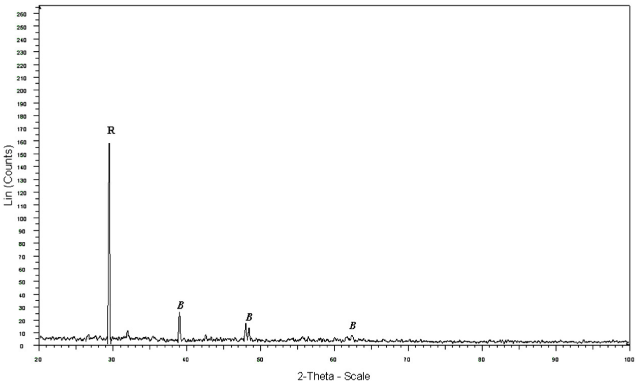

Fig. 2. XRD pattern of the Fe(III)-doped TiO_2 sample; R: rutile; B: β-TiO_2; Calcination temperature: 500±50°C; Fe/Ti = 0.034 at.%.

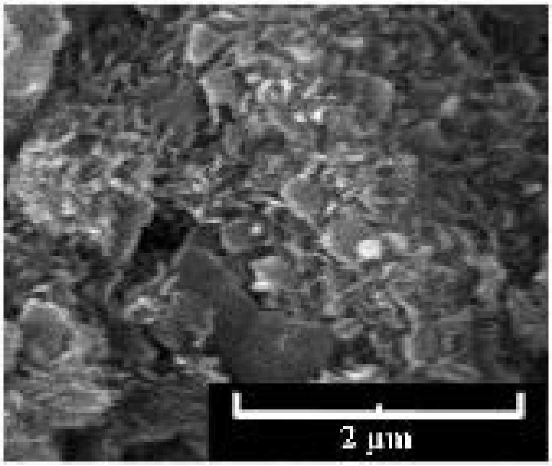

Fig. 3. SEM images of the Fe (III)-doped TiO_2 sample; SEM MAG: 25.05 kx; DET: SE Detector; HV: 25.0 kV.

·OH radicals attack to phenyl ring, yielding catechol, resorcinol and hydroquinone; then the phenyl rings in these compounds break up to give short-chain organic acids such as maleic, oxalic, acetic, formic, and finally CO_2. They indicated that the main by-products of phenol degradation under ultraviolet illumination are similar, whether nanocatalyst (TiO_2) is present or not. Based on our results, the same by-products were measured with Fe (III)-doped TiO_2.

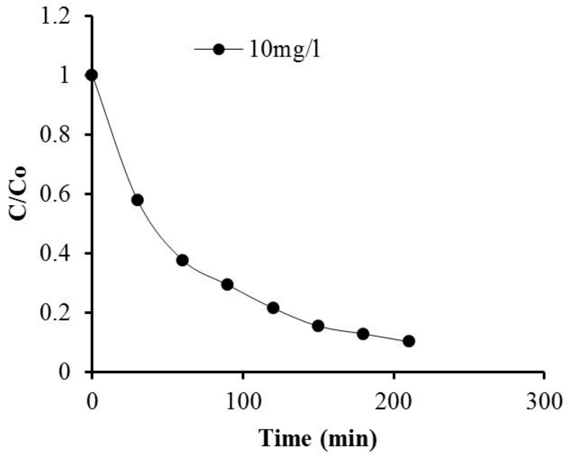

Fig. 4. Phenol degradation as a function of Fe (III)-doped TiO_2/UV; C_0 = 10 mg/L, pH = 3 and C_{cat} = 0.5 g/L.

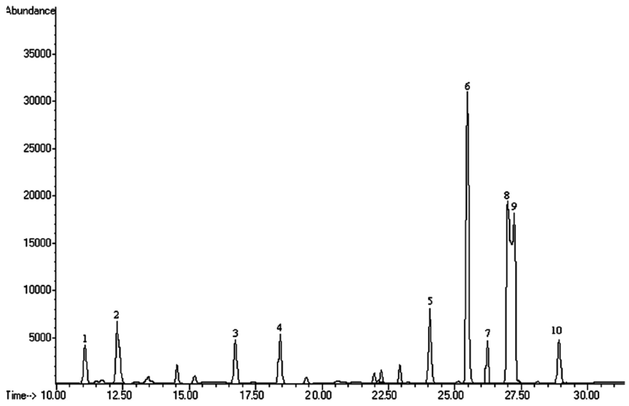

Fig. 5. By-products graph resulted from photocatalytic degradation of phenol, detected by GC-MS analysis after 210 min of UV illumination. The peaks in the graph corresponding to the organic compounds are: (1) Acetic acid; (2) Phenol; (3) Oxalic acid; (4) Glutaric acid; (5) Glycerol; (6) Catechol; (7) E-2- Butenedioic acid; (8) Resorcinol; (9) Hydroquinone; (10) 1,2,3-Benzenetriol.

According to the obtained values of LC_{50}s (Table 1), the toxicity of effluent was 6–9 fold higher than that of phenol solution at all of the exposure times. In fact the by-products introduce more toxic effects on *Daphnia magna*. Results of the present study confirmed that the Fe (III)-doped TiO_2/UV process is a good method for phenol removal, but more longer treatment is needed for complete degradation of phenol to final non-hazardous products and this phenomenon should be considered in the future applications.

Results of the LC_{50} and TU for initial phenol solution and the process effluent by *Daphnia magna* in exposure times of 24 h, 48 h, 72 h and 96 h are presented in Table 1. Based on these data and as it is shown in Figs. 6 and 7, the LC_{50} for initial phenol solution and the effluent at different times did not show a specified increasing or decreasing trend. In fact, the difference between the LC_{50} at different times was not statistically significant (P-value > 0.05). 24, 48, 72 and 96 h LC_{50} (V/V_0) values were 110.82, 91.47, 77.016 and 63.53 for initial phenol solution and 91.0034, 67.0213, 43.7498 and 33.1536 for the process effluent, respectively. An increase in toxicity of the process effluent in comparison with the initial solution of phenol is evident (Figs. 6 and 7), which can be attributed to the formation of by-products (see Fig. 5).

This result is not in accordance with previously found data on the sonochemical degradation of phenol [5]. According to [5] the TU value for the effluent was 1.21 times lower than that obtained for phenol solely and it was concluded that the toxicity of by-products formed during the sonochemical degradation of phenol is lower than the toxicity of phenol. The main differences between study [5] and

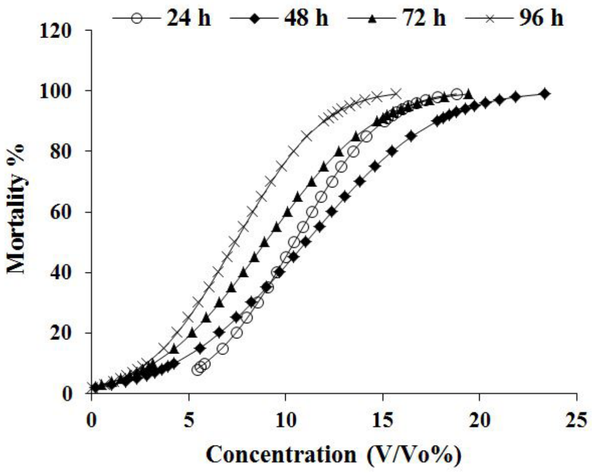

Fig. 6. Percentage mortality of *Daphnia magna* on exposure to phenol solution.

Table 1
Toxicity data for phenol solution and photocatalysis effluent

Test Sample	Initial phenol solution				Phenol degradation effluent			
Time (h)	24	48	72	96	24	48	72	96
LC_{50} (V/V_o%)	10.44	11.038	8.951	7.396	1.203	1.214	1.212	1.125
Toxicity unit (TU)	9.578	9.060	11.171	13.520	83.125	82.372	82.508	88.888

Phenol degradation effluent was obtained using the Fe(III)-doped TiO_2/UV process (210 min).

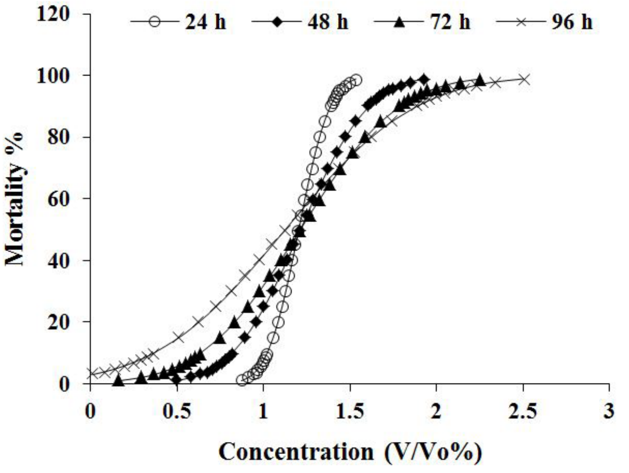

Fig. 7. Percentage mortality of *Daphnia magna* on exposure to effluent mixture.

the present study are the method and parameters of degradation which can affect the toxic metabolites production. In the present study hydroquinone and catechol were the two main toxic by-products. Hydroquinone induces karyotypic effects or chromosome aberrations in eucaryotic cells [23]. Some researchers have previously approved the higher toxicity of hydroquinone compared to phenol [24]. Also, 48 h LC50 of catechol on *Daphnia magna* was reported to be 1.09 mg/L which was close to phenol LC50 (0.91 mg/L) [25].

Increment in effluent toxicity of Fe (III)-doped TiO_2/UV process to *Daphnia* cannot be related to Fe (III)-doped TiO_2 nanoparticles, because previous investigation has shown that Fe(III)-doped TiO_2 nanoparticles synthesized by sol-gel method were less toxic for *Daphnia magna* in comparison with TiO_2 nanoparticles [14]. They reported an LC50 of more than 20,000 mg L^{-1} for Fe (III)-doped TiO_2 on *Daphnia magna* [14].

They reported an LC_{50} of more than 20,000 mg/L for Fe (III)-doped TiO_2 on *Daphnia magna* [14]. This value is very lower than concentrations that were used in the present study (500 mg/L). But some other researchers have shown that application of nanoparticles with other toxicants in the environment can cause increment in the toxicity of toxicants [26]. The mechanism of this toxicity increment could be explained through a two-phased adsorption/desorption process. In the first phase, where the concentration of toxicant is high in the nanoparticle surrounding solutions, nanoparticles adsorb toxicants (thus subtracting it from solution and making it less bioavailable). But when they enter the microorganism body or when they are in close proximity to microorganism, where the concentration of phenanthrene is low in the nanoparticle surrounding environment, in a second phase, toxicant is released and would become bioavailable to the affected microorganisms such as *Daphnia*. This mode of action has been considered as Trojan horse effect, which may enhance the toxicity and affect the fate, transformation, and transport of pollutants in the environment [26,27].

In this research, in order to better comparison of toxicity results, some parameters such as pH, temperature, and soluble ions were regarded according to standard method [16]. Such parameters can affect the toxicity results in real conditions.

The results highlight the necessity for addressing by-products problems of phenol degradation using Fe (III)-doped TiO_2or other catalysts nanoparticles in the photocatalytic process, disposal legislations, and design of standard criteria. But to make a definite conclusion, more researches on photocatalytic process are needed using different nanoparticles, bioandicators and toxicants.

4. Conclusion

Bioassay test of phenol solution and Fe (III)-doped TiO_2/UV process effluent was performed using *Daphnia magna*. Results indicated that the process effluent was more toxic than initial phenol solution.

GC–Ms results of effluent showed that the main by-products of phenol degradation under Fe (III)-doped TiO_2/UV process include hydroquinone, catechol, resorcinol, glycerol, glutaric acid, oxalic acid, 1,2,3–benzenetriol, phenol, acetic acid and E–2–butenedioic acid. Results of the present study confirmed that the Fe (III)-doped TiO_2/UV process may be a good method for phenol removal, but higher toxicity potential of the effluent showed that this process at our studied conditions is not adequate for complete degradation of phenol to final non-hazardous products and this phenomenon should be considered in the future applications and also in order to assess of effects of every single by-products of phenol on the toxicity, a future research is needed.

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