Modeling photocatalytic degradation of diazinon from aqueous solutions and effluent toxicity risk assessment using *Escherichia coli* LMG 15862

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Abstract

In this study, modeling and degradation of diazinon from contaminated water by advanced oxidation process together with a new test for effluent bioassay using *E. coli* were investigated. The experiments were designed based on response surface methodology. Nanoparticles (NPs) were synthesized using the sol–gel method. The shape characteristics and specifications of elements in the nanoparticles were characterized using scanning electron microscope and energy dispersive X-ray, respectively. Diazinon was measured using high performance liquid chromatography device and by-products due to its decomposition were identified by gas chromatography-mass (GC–MS). In the present study, effluent bioassay tests were conducted by defining the rate of dehydrogenase enzyme reducing alamar blue method. According to statistical analyses ($R^2 = 0.986$), the optimized values for pH, dose of NPs, and contact time were found to be 6.75, 775 mg/L, and 65 min, respectively. At these conditions, 96.06% of the diazinon was removed. Four main by-products, diazoxon, 7-methyl-3-octyne, 2-isopropyl-6-methyl-4pyrimidinol and diethyl phosphonate were detected. According to the alamar blue reducing (ABR) test, 50% effective concentration, no observed effect concentration, and 100% effective concentration ($EC_{100}$) for the mortality rate of *E. coli* were obtained as 2.275, 0.839, and 4.430 mg/L, respectively. Based on the results obtained, it was found that mentioned process was high efficiency in removing diazinon, and also a significant relationship between toxicity assessment tests were obtained ($P < 0.05$).

Keywords: Modeling, Diazinon, Dehydrogenase enzyme, Effluent bioassay

Introduction

Organophosphate pesticides (OPs) are among the largest and most diverse types of available pesticides. Considering that they affect a wide range of insects and rodents, these pesticides are used by farmers more than other types. But due to the lack of familiarity with the damaging effects of these toxins or proper principles of combating pests, most consumers do this job either incompletely or indiscriminately (Fadaei et al. 2012; Li et al. 2015; Maddah and Hasanzadeh 2017). Therefore, intentional or unintentional human exposure is as a result of the use of pesticides or their residuals in environments including air, water, soil, and plants. Considering global statistics, the largest portion of mortality from pesticides is related to these toxins. Diazinon is an organophosphate pesticides with $pK_a = 2.6$ and medium risk (Kalantary et al. 2014). The major effects of diazinon on vertebrate life are inhibition of acetyl cholinesterase, resulting in aggregation of acetylcholine in acetylcholine receiver and hyper excitation of nerves and muscles. So far, various technologies have widely been applied for removal of diazinon in aqueous solution such as adsorption, electrocoagulation...
and biodegradation (Amooey et al. 2014; Ehrampoush et al. 2017).

Since conventional water and wastewater treatment processes are not very effective on the degradation of diazinon (Amooey et al. 2014; Kalantary et al. 2014; Ehrampoush et al. 2017), Recently, advanced oxidation process such as UV/H₂O₂, H₂O₂/Fe²⁺, NPs/UV and etc., due to high efficiency, low cost, and non-toxicity have been considered. Li et al. used UV and UV/H₂O₂ process for the removal of diazinon from water resources (Li et al. 2015). Also, Kalantary et al. successfully used TiO₂/UV process for the degradation of diazinon (Kalantary et al. 2014). TiO₂ nanoparticles along with UV, have been considered as an effective method for water treatment (Amooey et al. 2014; Li et al. 2015; Ribeiro et al. 2015; Ehrampoush et al. 2017; Maddah and Hasanzadeh 2017). The energy of light from UV rays in contact with titanium atoms, stimulates its surface electrons and moves them from the valence layer to the conductive layer. The result of this energy change will be the formation of a halo at the surface of the titanium atom and the formation of free electrons (OH·). These active radicals cause oxidation of organic matter in the solution and convert it to water and carbon dioxide. One of the disadvantages of titanium nanocatalysts is the existence of an inter-structural hole in this composition. This means that a less energy band of ultraviolet radiation will remain on the surface of the catalyst (Mohammadi and Sabbaghi 2014; Tian et al. 2014; Toolabi et al. 2017; Wang and Shih 2016). Accordingly, in the current study, to enhance the optimal response of titanium dioxide, silica dioxide was introduced to the reaction. Performing the effluent toxicity risk assessment after water treatment processes is essential for environmental, drinking water and public health. Previously, to determine the effluents toxicity, some methods such as tetrazolium salt, crystal violet, and colony forming unit were used. But often they were expensive, long-term, and unreliable (Pettit et al. 2005; Satyanarayan et al. 2016). Recently, alamar blue (AB) due to its high sensitivity and non-toxicity has been widely used in studies on bioassay in a biological range such as bacteria, piscine cells and planktonic assays (Rampersad 2012; Khalifa et al. 2013; Teh et al. 2017).

Because the oxidation–reduction potential (ORP) of alamar blue is more than the enzyme dehydrogenase, it was reduced by the dehydrogenase enzyme. But in the presence of living bacteria, alamar blue is converted to resorufin and the color of the solution changes from blue to pink (Nasiry et al. 2007; Rampersad 2012; Gregoraszczuk et al. 2015; Balouiri et al. 2016; Tyc et al. 2016; Zare et al. 2016; Teh et al. 2017; Toolabi et al. 2017). To achieve the best and most effective method of removal and risk assessment of diazinon in aqueous solution, more studies need to be done in this regard. Therefore, in this study, application of Fe₃O₄/SiO₂/TiO₂/H₂O₂/UV-C process for the degradation of diazinon and novel test for the effluent toxicity risk assessment using Escherichia coli were conducted.

**Materials and methods**

**Chemicals and media**

Analytical diazinon pesticide with a purity of 98.5%, Acetic acid 99.9%, ethanol 99.9%, chloride iron (II), chloride iron (III), tetra ethyl ortho silicate 95%, tetra-n-butyl lorthotitanate, ammonium solution, alamar blue powder, agar muller hinton, broth nutrient, dimethyl sulfur oxide (DMSO), n-amyl alcohol, HCl-phthalate buffer, glucose, sodium acetate, sodium bicarbonate, Sulfuric acid 98%, Sodium Hydroxide 98%, potassium phosphate monobasic and Dipotassium phosphate were purchased from Sigma Aldrich Co. The properties of diazinon and alamar blue are shown in Table 1.

**Microorganism**

A standard strain of Escherichia coli LMG 15862 bacteria was purchased from Tehran Razi Institute and immediately was stored at a temperature of 8 °C.

**Synthesis of nanoparticles**

There are various methods for synthesizing and doping TiO₂/Fe₃O₄/SiO₂ nanoparticles. These routes include sol–gel process, co-precipitation, hydrothermal method, pyrolysis spray, sono-chemical synthesis, and wet immersion method (Tian et al. 2014; Gupta et al. 2015).

**Fe₃O₄/SiO₂**

The synthesis of Fe₃O₄ nanoparticles was done according to co-precipitation method. Briefly, 23.36 g of chloride iron (III) and 8.62 g of chloride iron (II) were dissolved in 250 cc of deionized water for 50 min and mixed at 87 °C inside a reactor (Cylindrical and quartz glass with a diameter of 35 cm and length of 45 cm). Thereafter, the resulting solution was slowly injected into 3.6 L of deionized water. Next, action bubbling of nitrogen gas was conducted for 24 h at 75 °C. After these three stages of washing with water and ethanol, Fe₃O₄ nanoparticles were formed (Shunxing et al. 2016; Maddah and Hasanzadeh 2017; Toolabi et al. 2017).

**Fe₃O₄/SiO₂/TiO₂ nanoparticles**

The synthesis of nanoparticles was done using the sol–gel method. The nanoparticles obtained in the previous step were dissolved in 250 cc deionized water containing tetraethyl orthosilicate, in the next step, ultrasonic (Hielscher model, Sonication of liquids 0.5–4.0 L/min) was used to better separate the nanoparticles. Thereafter,
for transparency of nanoparticles and crystal formation, 30 mL of acetic acid was added to the reactor containing nanoparticles of iron/silica and mixed at 200 rpm. Next, the combination of acetic acid, ethanol and tetra-n-butyl lorthotitanate was prepared. The mixture obtained was added to the heater reactor and mixed at 500 rpm. After three stages of washing with deionized water and ethanol, Fe₃O₄/SiO₂/TiO₂ was formed (Shunxing et al. 2016; Toolabi et al. 2017; Wang et al. 2017). The surface and shape characteristics of the nano composite and quantitative analysis of the elements were characterized using a scanning electron microscope and energy dispersive X-ray, respectively.

**Modeling and statistical analysis**

In this work, to model and design the experiments, response surface methodology (RSM) was used. This model is a collection of statistical and mathematical techniques that are useful for analyzing the effects of several independent variables on a response. RSM is an effective statistical technique for optimizing the number of experiments. Also, it specifies the interconnected amount of variables and the most optimal variable is presented in order of preference. RSM contains various models such as the Behnken design, central composite design (CCD), factorials method, box-d-optimal design etc. (Martino et al. 2015; Sarrai et al. 2016; Dehghani et al. 2017; Nama et al. 2018). In the present study, according to the CCD model, the number of experiments was designed for variables such as diazinon concentration (1–40 mg/L), contact time (10–120 min), pH (3–12), and dose of nanoparticles (100–1000 mg/L), Table 2. Expert Design Ver 7 was used for the data analysis.

| Table 1 Properties of diazinon and alamar blue |
|-----------------------------------------------|
| **Diazinon**                                  |
| Chemical formula: C₁₂H₂₁N₂O₃PS                |
| Molekulargewicht: 304.35 g/mol                |
| Density: 1.17 g/cm³                           |
| Half-life in neutral water: 138 day           |
| Half-life in soil: 37.4 day                   |
| Solubility in water: 40 mg/L at 25 °C         |
| Vapor pressure: 1.4 × 10⁻⁴ mm Hg at 20 °C     |
| Henry’s law constant: 1.4 × 10⁻⁶ atm³/mol     |
| **Alamar blue**                               |
| Chemical formula: C₁₂H₇NO₄                    |
| Solubility in water: soluble                 |
| Molar mass: 229.19 g/mol                     |

| Table 2 The levels of variables central composite statistical experiment design |
|------------------------------------------|----------------|----------------|--------|--------|
| Factor | Variables                      | Low actual | High actual | Mean  | Std. Dev. |
| A      | pH                             | 2.012      | 7.500       | 9.75  | 5.25     |
| B      | Contact time (min)             | 24.597     | 65.000      | 92.50 | 37.50    |
| C      | Concentration of diazinon(mg/L) | 8.721      | 20.500      | 30.25 | 10.75    |
| D      | Dose of NPs (mg/L)             | 201.246    | 550.000     | 775.00| 325.00   |
Analytical procedures
Experiments were conducted inside a glass reactor (11 × 11 × 25 cm) with a reflective wall. This reactor was equipped with a UV lamp (λ = 254 nm, P = 125 W, L = 10 cm) surrounded by a quartz tube, cooling system, an air blower pump with a flow rate of 3 L per minute to remove gases from the reactor and also to prevent possible precipitation of nanoparticles to the bottom of the reactor, pH meter and multiparameter device. Also a radiometer device (model Hanger ECL-X) was used to measure the intensity of UV radiation. During the experiment process, sampling was done based on the CCD. In order to increase the production of radical hydroxyl ion in a solution, H$_2$O$_2$ compound was used at a concentration of 50 mg/L (Shemer and Linden 2006). All samples filtered by using a syringe equipped with a 0.2 micron filter. The concentration of diazinon was measured using High Performance Liquid Chromatography (HPLC), the following specifications were used; wavelength was 260 nm, C18 column, length and diameter of the column were 4.6 × 250 mm and the volume of injection sample = 20 µL. The removal efficiency of diazinon was obtained using the following Eq. 1.

\[
\text{Removal} (%) = \left(1 - \frac{C_t}{C_0}\right) \times 100
\]

where $C_t$ is the initial concentrations of diazinon (mg/L) and $C_t$ is the residual of diazinon (mg/L) after the specified time.

By-products resulting from the degradation of diazinon were detected using gas chromatography–mass (GC–MS) model Agilent Technologies 19091S-433 with a HP-5MS column (length 25 m, thickness 0.25 mm, diameter 0.25 mm) (Ehrampoush et al. 2017; Toolabi et al. 2017).

Chemical oxygen demand (COD)
Based on the standard methods in the purification of water sources, the rate of mineralization of diazinon was determined by measuring the COD. Accordingly, COD removal was determined using Eq. 2.

\[
\%\text{COD Removal} = \left(\frac{\text{COD}_{in} - \text{COD}_r}{\text{COD}_{in}}\right)
\]

where $\text{COD}_{in}$ is initial COD (mg/L) and $\text{COD}_r$ is the COD (mg/L) residual concentration according to CCD parameters.

Toxicity assessment based on ABR methods
The rate of alamar blue dye reduction was determined by the activity of enzyme dehydrogenase; first broth nutrient culture medium was enriched with KH$_2$PO$_4$ (3.28 g/L), K$_2$HPO$_4$ (5.28 g/L), sodium acetate (0.4 g/L) and glucose (0.4 g/L). Next, 2 mL of $E.\ coli$ suspension and 2 mL of alamar blue solution with concentration of 200 mg/L were added to the broth nutrient medium. Then, 1 mL of the diazinon was added with specific concentrations. Next, it was incubated at 30 °C under darkness condition. Following 60 min of contact time, 2 mL of HCl-phthalate 0.05 M buffer and 20 mL of n-amyl alcohol solution were added to each test tube. Afterwards these materials were stirred slowly. The rate of alamar blue reduction was determined through the extent of absorption at the wavelength of 620 nm using UV/ViS spectrophotometer device (Braic 2100) (Toolabi et al. 2017; Zare et al. 2016). The percentage of alamar blue reduction was obtained using Eq. 3.

\[
\text{Reduce activity of dehydrogenase enzyme in alamar blue conversion} = \left(\frac{A - B}{A}\right) \times 100
\]

where A is the rate of activity of dehydrogenase enzyme in alamar blue conversion in the control sample and B is the rate of activity of dehydrogenase enzyme in the main sample.

Toxicity assessment based on CFU methods
To investigate the validity of ABR test and effluent bioassay, CFU test was conducted. Accordingly, first, a suspension of $E.\ coli$ LMG bacteria was prepared. Suspension turbidity was detected using spectrophotometer device. Based on 0.5 McFarland, optical density (OD) 0.6 was generated. By measuring the turbidity in the suspension, the density of the bacterial cells was obtained in the range of 2–3 × 10$^8$ cells/mL. To determine the mortality rate of $E.\ coli$ bacteria, 100 µL of bacterial suspension was injected on a plate containing the Mueller–Hinton medium and diazinon (Nasiry et al. 2007; Gregoraszczuk et al. 2015; Balouiri et al. 2016; Tyc et al. 2016; Toolabi et al. 2017). After 24 h of incubation, the growth inhibition percentage was determined by Eq. 4.

\[
\text{Growth inhibition percentage} = \frac{A - B}{A} \times 100
\]

where A is the number of colonies of the control sample and B is the number of colonies of the inoculated sample. Finally, for both tests (ABR and CFU), the results were reported as follows: The amount of toxin required for decreasing the growth less than 1% of the bacteria initial population was reported as no observed effect concentration (NOEC), the amount of toxin required for decreasing 50 and 100% of the bacterial growth was reported as effective concentration (EC$_{50}$) and effective concentration (EC$_{100}$), respectively.

Sampling from natural source
After determining the optimal parameters for removal of diazinon by advanced oxidation process, sampling of water from the Seymareh River was carried out for 6
consecutive months. Sampling was carried out once a week and the volume of each sample 2 L was selected. After the samples were transferred to the laboratory, their physical and chemical characteristics were determined. Samples were introduced into the photocatalyst reactor. And removal efficiency of diazinon were obtained under optimum conditions. Then, Alamar Blue reduction and colony count unite tests were used to determine the toxicity of the effluent.

Results

Scanning electron microscopy
Information on surface morphology and particle size distribution of Fe₂O₄ and Fe₂O₄/SiO₂/TiO₂ were characterized using Scanning electron microscopy, Fig. 1. Accordingly, the high transparency of nanoparticles production was achieved with energy of 15 kV and their accumulation properties were not observed. Also, according to the analysis of the size of the nanoparticles, the typical size of nanoparticles was determined to be 200 nm.

Energy dispersive X-ray spectroscopy
According to Fig. 2, elemental composition analysis using EDX was presented at 0.2 to 8 keV. In Fe₂O₄/SiO₂ composite, O, Fe, Si, and S elements were diagnosed Fig. 2a. The weakest and strongest signals were related to S and Fe elements, respectively. It was also shown in Fig. 2b that Fe₂O₄/SiO₂/TiO₂ nanoparticles contain O, C, Fe, Si, Ti, S, and Cr elements. The weakest and strongest signals were related to Cr and O, respectively.

Statistical analysis and modeling
According to the central composite design, the number of 30 runs was designed and the efficiency removal of diazinon belonging to each run was determined, Table 3. The optimum run was related to run 27; in this case, the removal efficiency of diazinon was reported to be 96.06%. Also, the predicted value of each run was determined. Accordingly, a direct relationship between real values and predicted values was reported Fig. 3 (R² = 0.943). Further details are shown in Table 3.

In this study, the regression results of quadratic, linear, 2FI, and cubic models for the removal efficiency of diazinon is shown in Table 4. Accordingly, for R² = 0.9865, the quadratic model was more credible than other models. The final equation to describe the actual factors according to the quadratic model is shown in Eq. 5.

Removal efficiency of diazinon%

\[ = 89.26 - 3.528 \times A - 0.3342 \times B - 3.574 \times C - 3.023 \times D - 0.2713 \times AB - 0.1875 \times AC - 0.3050 \times AD - 0.3262 \times BC - 0.3988 \times BD - 0.3075 \times CD - 4.496 \times A^2 - 2.586 \times B^2 - 0.3646 \times C^2 - 1.360 \times D^2 \] (5)

Based on Eq. 5, the maximum removal percentage of diazinon 96.06 was obtained. Impact coefficient for variables such as pH, contact time, diazinon concentration, and dose of NPs was obtained 3.528, 0.3342, 3.574 and 3.023, respectively. As shown in Eq. 5, the main parameter

![Fig. 1](The results of SEM images of a Fe₂O₄ nanoparticles and b Fe₂O₄/SiO₂/TiO₂ nanoparticles)
is related to the pH variable. Also, the minimum and maximum interaction amount variables in relation to the coefficient of AC and BD Coded Factors were obtained as 0.1875 and 0.3988, respectively. In this study, the F-value, P value and degree of freedom (DF) parameters were conducted for the analysis of variance. According to the results shown in Table 5, the F-value, P-value and DF were obtained as 78.32, < 0.0001, and 14, respectively.

**Effect of variables on the removal efficiency**

The results indicated that this process has been highly efficient in the removal of diazinon and COD. As shown in Figs. 3, 4, D response and contour plot models were studied for the removal of diazinon. The effect of the initial concentration of diazinon in the reactor was investigated from 1 to 40 mg/L. As shown in Fig. 4, by increasing the initial concentration of diazinon, the removal efficiency decreased. Accordingly, at pH = 6.75 and contact time = 65 min, by increasing the initial concentration from 10.75 to 30.25 mg/L, removal efficiency of diazinon decreased from 92 to 85%. The optimal pH for diazinon removal was obtained near 7. When pH increased from 6.75 to 9.5, the removal efficiency of diazinon decreased from 90.5 to 82%. Also, in this study, optimal contact time and optimal dose of nanoparticles were obtained in 65 min and 775 mg/L, respectively, Fig. 4.

**Identification of products by GC–MS**

In this study, the analysis of by-products was performed based on the following conditions; pH = 6.75, contact time = 40–80 min, dose of NPs = 775 mg/L and diazinon Concentration = 10.75 mg/L. Speciation and molecular
structures of the oxidation by-products were analyzed by GC–MS, Fig. 5. According to the results shown in Table 6, four by-products, including: diazoxon, 7-methyl-3-octyne, 2-isopropyl-6-methyl-4-pyrimidinol (IMP) and diethyl phosphonate were identified during degradation of diazinon. Their retention time (RT) varied from 2.15 to 15.75 min. As such, the minimum and maximum RT were related to diazoxon and diethyl phosphonate compounds, respectively. The characteristics of other compounds are shown in Table 6.

**Effluent toxicity assessment**

In this study, to determine the mortality rate of *E. coli* LMG bacterial, NOEC, effective concentration (EC) parameter was used. Accordingly, the growth inhibitory level Before and after from performing the advanced
oxidation process (AOP) was obtained. EC_{50} related to ABR and CFU tests before from performing AOP was obtained as 2.255 and 2.250 mg/L, respectively, Also, NOEC related to ABR and CFU tests was obtained as 0.890 and 0.850 mg/L, respectively, Table 7. Based on the results shown in Table 8, the effluent toxicity assessment from the reactor in different runs for EC_{50} and NOEC parameters related to ABR test after from performing AOP were obtained as 2.275 and 0.839 mg/L, respectively.

Analysis of the river water samples

The characteristics of raw water of Seymareh Rive are shown in Table 9. Based on the results of analysis of the River water samples, it was found that the removal efficiency of diazinon by advanced oxidation process is 95%, and it was found that the COD was decreased from 55 to 1.65 mg/L. By analyzing the effluent toxicity Using Alamar blue and colony forming unit tests, it was observed that the number of bacteria are not decreased.

Discussion

According to the results obtained in Fig. 1, it was found that the syntheses of Fe_{3}O_{4}/SiO_{2}/TiO_{2} nanoparticles were successful. By using SEM techniques, the size of the nanoparticles was confirmed at a range of 200 nm. Also, by comparing the elements and peaks produced by EDX analysis, It was found that sol–gel and co-precipitation methods were acceptable for the synthesis of nanoparticles in this study.

According to the analysis of variance Table 5, values of Prob > F less than 0.0500 show that the model quality is significant. Accordingly, the A, C, D, A_{2}, B_{2}, and D_{2} parameters are significant. The F value of 78.32 and the Prob > F value of < 0.0001 suggest that the model was statistically approved for removal of diazinon. Also, based on the results obtained from the quadratic model in Table 4, the R^{2} value and Adj R^{2} value were obtained as 0.986 and 0.973, respectively. These results showed that the predicted values obtained from the quadratic model is a fit of the experimental results (Martino et al. 2015; Sarrai et al. 2016; Dehghani et al. 2017).

In order to increase the photo catalytic properties in the process, hydrogen peroxide was added to the reactor. Hydrogen peroxide led to more formation of hydroxyl radicals and resulted in the oxidation of the pesticide compounds (Fadaei et al. 2012; Asaithambi et al. 2017). According to the results obtained in Fig. 4, by increasing the contact time from 37 to 65 min, removal efficiency of diazinon was increased from 85.5 to 91%. This is due to the production of more OH radicals in longer time and also more exposure of active radicals by diazinon; the possibility of the decomposition of a larger percentage of diazinon is provided. Based on the results from the one factor response model, three-dimensional response and contour model, by increasing the concentration of NPs, the removal efficiency of diazinon was increased.
Therefore, at a dosage of 320 mg/L of NPs, the removal percentage of diazinon was obtained at 85. Once the dosage of NPs was increased to 775 mg/L, the removal percentage of diazinon reached 92.5. This is because, when the concentration of nanoparticles under the influence of UV radiation is increased in the reactor test, $h^+$ and $e^{-}$ ions are produced. Afterwards, these ions react with water and peroxide radicals and also hydroxide ions are produced (Shunxing et al. 2016; Toolabi et al. 2017). Peroxide radicals are mixed with $H^+$ ions and hydroxyl radicals (OH•) are formed. Due to the high oxidation power of OH radicals, the degradation of the diazinon occurred. In this study, it was found that due to the reflective wall of the reactor, the amount of radiation produced is 1.45 times higher than that of conventional reactors under similar conditions. This causes more electrons to be stimulated from the catalyst surface, and the production of active radicals in the solution increased.

Based on the results of this study, pH was the most effective parameter in removing diazinon. The maximum removal efficiency was obtained when pH was equal to 6.75, this was because more hydrolysis of diazinon occurs in acidic solutions. Also, the production of active hydroxyl radicals is higher in acidic solutions. Therefore, this parameter should be given more attention in future studies (Li et al. 2015; Ehrampoush et al. 2017; Toolabi et al. 2017). In the study of Kalantary et al., optimal parameters such as pH of the nanoparticle and the contact time degradation of diazinon were obtained by using the TiO$_2$/UV process at 6, and 550 mg/L, and 60 min.

Table 7 The result of diazinon effect concentration in ABR and CFU tests by using E. coli

| Parameters | Type of test | Bottom limit | Upper limit | Typical value |
|------------|--------------|--------------|-------------|---------------|
| EC$_5$ (mg/L) | ABR | 0.316 | 0.329 | 0.324 |
| CFU | 0.313 | 0.323 | 0.319 |
| EC$_10$ (mg/L) | ABR | 0.519 | 0.533 | 0.530 |
| CFU | 0.515 | 0.529 | 0.525 |
| EC$_15$ (mg/L) | ABR | 0.817 | 0.829 | 0.823 |
| CFU | 0.816 | 0.825 | 0.820 |
| EC$_20$ (mg/L) | ABR | 0.959 | 1.000 | 0.968 |
| CFU | 0.950 | 0.987 | 0.964 |
| EC$_25$ (mg/L) | ABR | 1.287 | 1.297 | 1.293 |
| CFU | 1.272 | 1.287 | 1.285 |
| EC$_30$ (mg/L) | ABR | 1.502 | 1.513 | 1.510 |
| CFU | 1.489 | 1.508 | 1.500 |
| EC$_35$ (mg/L) | ABR | 1.612 | 1.630 | 1.619 |
| CFU | 1.609 | 1.621 | 1.611 |
| EC$_40$ (mg/L) | ABR | 1.826 | 1.831 | 1.826 |
| CFU | 1.812 | 1.852 | 1.818 |
| EC$_45$ (mg/L) | ABR | 2.030 | 2.041 | 2.034 |
| CFU | 2.022 | 2.032 | 2.027 |
| EC$_50$ (mg/L) | ABR | 2.230 | 2.265 | 2.255 |
| CFU | 2.235 | 2.255 | 2.250 |
| EC$_55$ (mg/L) | ABR | 2.360 | 2.372 | 2.363 |
| CFU | 2.339 | 2.351 | 2.347 |
| EC$_60$ (mg/L) | ABR | 2.584 | 2.591 | 2.587 |
| CFU | 2.545 | 2.577 | 2.571 |
| EC$_65$ (mg/L) | ABR | 2.719 | 2.732 | 2.724 |
| CFU | 2.761 | 2.795 | 2.790 |
| EC$_70$ (mg/L) | ABR | 2.932 | 2.961 | 2.951 |
| CFU | 2.900 | 2.920 | 2.911 |
| EC$_75$ (mg/L) | ABR | 3.100 | 3.125 | 3.111 |
| CFU | 3.127 | 3.149 | 3.145 |
| EC$_80$ (mg/L) | ABR | 3.329 | 3.343 | 3.335 |
| CFU | 3.351 | 3.371 | 3.360 |
| EC$_85$ (mg/L) | ABR | 3.742 | 3.659 | 3.662 |
| CFU | 3.668 | 3.702 | 3.689 |
| EC$_90$ (mg/L) | ABR | 3.878 | 3.888 | 3.881 |
| CFU | 3.890 | 3.823 | 3.812 |
| EC$_95$ (mg/L) | ABR | 4.000 | 4.116 | 4.080 |
| CFU | 3.975 | 4.120 | 4.020 |
| NOEC (mg/L) | ABR | 0.884 | 0.893 | 0.890 |
| CFU | 0.847 | 0.859 | 0.850 |
| EC$_100$ (mg/L) | ABR | 4.010 | 4.300 | 4.128 |
| CFU | 3.980 | 4.221 | 4.120 |

Table 6 The characteristics of by-products identification due to diazinon decomposition

| Compound name | Molecular formula | Retention time (min) | Molecular weight (g/mol) |
|---------------|-------------------|----------------------|--------------------------|
| Diazoxon      | C$_{12}$H$_{21}$N$_{2}$O$_{4}$P | 2.15                 | 288.284                  |
| 2-isopropyl-6-methyl-pyrimidin-4-ol (IMP) | C$_{9}$H$_{12}$N$_{2}$O | 3.25                 | 152.197                  |
| 7-Methyl-3-octyne | C$_{7}$H$_{15}$ | 7.65                 | 124.223                  |
| Diethyl phosphonate | C$_{4}$H$_{10}$O$_{3}$P$^+$ | 15.75                 | 137.095                  |
respectively and the maximum removal efficiency of diazinon was obtained as 71% (Kalantary et al. 2014). This difference in the removal of diazinon can be due to the experimental conditions, such as the presence of silica and hydrogen peroxide in the present study.

According to the results of this study, four by-products, diazoxon, 7-methyl-3-octyne, 2-isopropyl-6-methyl-4-pyrimidinol (IMP) and diethyl phosphonate were identified during degradation of diazinon. By increasing the contact time from 40 to 80 min, the major of by-products were disappeared. Also by determining the toxicity of the effluent from the reactor, it was found that the toxicity of these compounds was less than that of diazinon. Similar to this study (Li et al. 2015), IMP was reported as the oxidation product of diazinon during advanced oxidation process, which is less toxic than its parent compound.

Also, In another study conducted by Kalantary et al. (2014), diazoxon and IMP compounds were introduced as by-products due to the diazinon degradation and by assessing their toxicity, it was found that their toxicity is less than that of diazinon. Therefore, according to the

| Parameters             | Value (average) |
|------------------------|-----------------|
| Temperature (°C)       | 16.3            |
| Turbidity (NTU)        | 105             |
| Oxygen dissolve (mg/L) | 6.2             |
| BOD (mg/L)             | 35              |
| COD (mg/L)             | 55              |
| Concentration of diazinon (mg/L) | 1.17       |

Table 8 The result of COD Removal, ORP and bioassay test to determination of effluent toxicity in different Runs

| Run | COD removal (%) | ORP (mv) | Residuals of diazinon (mg/L) | Rate of EC, NOEC |
|-----|-----------------|----------|-----------------------------|------------------|
|     |                 |          |                             | ABR test         |
| 1   | 94.50           | 333      | 2.275                       | EC50             |
| 2   | 96.00           | 330      | 2.320                       | EC55             |
| 3   | 94.40           | 325      | 2.462                       | EC59             |
| 4   | 95.30           | 322      | 2.585                       | EC56             |
| 5   | 96.00           | 339      | 1.863                       | EC50             |
| 6   | 95.70           | 337      | 1.906                       | EC52             |
| 7   | 93.90           | 338      | 1.763                       | EC56             |
| 8   | 92.80           | 327      | 2.446                       | EC54             |
| 9   | 94.00           | 341      | 1.709                       | EC57             |
| 10  | 92.80           | 331      | 2.437                       | EC55             |
| 11  | 92.10           | –        | 7.169                       | EC100            |
| 12  | 88.90           | –        | 9.044                       | EC100            |
| 13  | 93.50           | –        | 5.683                       | EC100            |
| 14  | 90.00           | –        | 8.702                       | EC100            |
| 15  | 96.90           | 349      | 1.075                       | EC100            |
| 16  | 96.00           | 340      | 1.720                       | EC100            |
| 17  | 93.80           | 350      | 1.075                       | EC100            |
| 18  | 91.50           | 340      | 1.720                       | EC100            |
| 19  | 93.50           | –        | 5.142                       | EC100            |
| 20  | 92.00           | –        | 6.987                       | EC100            |
| 21  | 93.80           | –        | 5.142                       | EC100            |
| 22  | 92.00           | –        | 6.985                       | EC100            |
| 23  | 93.20           | –        | 4.434                       | EC100            |
| 24  | 85.00           | –        | 7.523                       | EC100            |
| 25  | 93.00           | –        | 4.430                       | EC100            |
| 26  | 94.00           | 301      | 4.305                       | EC100            |
| 27  | 99.20           | 360      | 0.839                       | NOEC             |
| 28  | 94.50           | –        | 8.396                       | EC100            |
| 29  | 92.40           | –        | 4.674                       | EC100            |
| 30  | 96.50           | 335      | 2.050                       | EC100            |

Table 9 Characteristics of raw water of Seymareh Rive
results obtained in this study, Fe$_2$O$_3$/SiO$_2$/TiO$_2$/H$_2$O$_2$/UV-C process by producing active radicals (OH$^*$) can decompose diazinon and its by-products.

According to the results from Table 8, the degree mineralization of diazinon was determined by using COD experiment. Therefore, the minimum and maximum mineralization of diazinon was determined by using the experiments; AE and MA made a substantial contribution to the analysis. MTG, MM and AT carried out experiments; AT conceived and designed the experiments; MTG wrote the paper. All authors read and approved the final manuscript.

Authors' contributions
MTG, MM and AT conceived and designed the experiments; AE and MA made a substantial contribution to the analysis and interpretation of the data presented; MHE and AT wrote the paper. MHE, AT, MM and MTG conceived and designed the experiments; AT performed the experiments; AE, MAS and MA made a substantial contribution to the analysis and interpretation of the data presented; MTG wrote the paper. All authors read and approved the final manuscript.

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