**Investigation of Trichlorfon pesticide degradation in water using UV/ SO32– advanced reduction process: Mineralization, intermediates detection and ECOSAR-based bioassay**

**Abstract**

This study aimed to investigate the degradability, mineralization, proposed decomposition pathway, intermediate products, and toxicity of effluent from trichlorfon (TCF) degradation in water by UV / sulfite –advanced reduction process (UV/S-ARP).

This study was experimentally performed in a photochemical reactor as a batch operation. The source of light was a UV lamp. Sulfite ion was used as the reducing agent. After the treatment, the residual concentration of TCF was measured by liquid chromatography equipped with tandem mass spectrometry (LC-MS/MS). UV/S-ARP had the highest performance at an initial pH of 7, a sulfite ion concentration of 120 mg/L, a contact time of 60 min, and a TCF concentration of 10 mg/L. Under such conditions, the degradation efficiency of TCF was 96.0 %, and the amount of mineralization based on the removal of TOC and COD were 74.6% and 79.5%, respectively, the results of the degradation mechanism showed that eaq- and SO3•- have played the greatest role in dechlorination and transformation of TCF. Based on the identified intermediates, more complex compounds are transformed into compounds with simpler structures by UV/S-ARP. Evaluating the toxicity of TCF by-products via ECOSAR bioassay showed that as-generated intermediates do not have acute and chronic adverse effects on fish. The results of our study indicated that the advanced reduction process could be an effective process for the purification of TCF-contaminated water.

1. **Introduction**

Organophosphorus pesticides, including trichlorfon (TCF), are widely used to manage pests effectively and increase crop yields. The presence of their residues in water sources has become one of the most important environmental issues worldwide. The physical and chemical properties of TCF caused its persistence and adverse effects in the environment. The solubility of TCF in water is high and will increase by increasing temperature. The compound is slowly hydrolyzed in an acidic medium and has a half-life of 526 days at a pH range from 1-5 and a temperature of 20 °C. TCF is rapidly converted to dichlorvos under alkaline conditions, a more toxic pesticide than the parent substance, and eventually converted to hydrolytic products. The presence of this pesticide in the environment and water resources can cause acute and chronic toxicity on humans and other living organisms. Long-term studies show that this compound can have mutagenic and defective effects on animal species as well as the human body.

Bioremediation, coagulation, liquid extraction, solid-phase extraction, adsorption, aerobic decomposition, electrochemical and biological oxidation, membrane technology (nano-filtration), and advanced oxidation processes such as homogeneous and heterogeneous photocatalysis were widely used to degrade pesticides in aquatic environments. Furthermore, in recent years, advanced oxidation, adsorption, and photocatalytic degradation methods using sunlight were used to decompose some pesticides and other refractory organic pollutants from water resources. Many of these methods of decomposing toxins only concentrate the water pollutants without destroying or removing them, and some of these methods have low efficiency in decomposing toxins. In recent years, ARPs as a new chemical treatment method were successfully utilized to convert target pollutants into simpler or harmless products. Moreover, partial degradation of refractory organic contaminants can lead to form the biodegradable intermediates (Liu et al., 2014). The most important advantage of ARPs over advanced oxidation processes is that they convert halogenated refractory organic into products with low toxicity at a high reaction rate (Liu et al., 2013). In recent years, Hofman-Caris et al. show that the ARPs gives good degradation of organic micropollutants in water and it is a possible alternative to AOPs such as the UV/H2O2 process. Moreover, ARPs were proposed as a new, simple, and economic method in the decomposition of certain of refractory organic compounds. Therefore, considering TCF pesticide is a resistant compound with chlorine bonds, ARPs as a simple, inexpensive, and environmentally friendly method can play an effective role in transforming and removing this pollutant. Hence, studies should be extended to evaluate the influence of various process parameters, mineralization efficiency, intermediates products, and toxicity assessment of intermediates products of this pesticide.

## **2. Material and Methods**

**2.1 Materials**

The analytical-grade TCF with a purity of more than 98%, sodium sulfite (purity >95%), sodium nitrite (purity >99%) was provided by Merck, Co (Germany). All contaminated waters with TCF and blanks were prepared with deoxygenated distilled water. Solutions were daily prepared. The sodium sulfite was added to the reaction vessel to supply the required sulfite ions.

**2.2 Experimental procedures**

This research was an experimental study, and an ARP was used to remove TCF in aqueous solutions on a laboratory scale. All tests were conducted on a cylindrical steel reactor with a diameter of 6 cm, the height of 24 cm, and a capacity of 500 ml. A UV lamp supplied the light source with a maximum wavelength at 253.7 nm in the UVC region. The solution with a volume of 200 mL containing TCF was added to the reactor and was mixed with sodium sulfite by placing the reactor on a magnetic shaker, and sampling was performed at different time intervals and analyzed according to standard methods (JIS 2017). Phosphate buffer was used to attain a stable pH during the experiments. The effects of important parameters influencing the degradation of TCF were also evaluated as batch experiments. These parameters include the initial concentration of TCF (2-4-6-8-10 mg/L), pH (5, 7, and 9), the amount of sulfite ion as a reducing agent (60-120-180 mg/L), reaction time (15-60 min), and UV intensity (540, 1091 and 1281 µW/cm2). LC-MS/MS analyzed initial and final concentrations of TCF. Water contaminated samples were also tested for the degree of dechlorination of TCF by this process. To investigate the reaction mechanisms, scavenging experiments were conducted, and the results were compared with the conditions in non-scavenging experiments. Chemical oxygen demand (COD) and total organic carbon (TOC) analyses were performed on the inlet and outlet of the treatment process to investigate TCF mineralization. Besides, the effect of UV and sulfite on the efficiency of ARP alone was analyzed. The production of intermediates was also analyzed using LC-MS/MS during sample analysis.

**2.3. Analytical methods**

Using one factor at a time methodology, 111 samples were analyzed in different environmental conditions. The concentrations of TCF, COD, TOC, and chlorine ions released in an aqueous solution were measured in the collected samples. The initial and final pH of the water solutions has also been measured to control the process.

TCF pesticide concentration was measured by Agilent LC 1200 HPLC (USA) coupled to an Agilent 6410 Mass Spectrometer triple quadrupole (QqQ) (Agilent technologies, USA) equipped with electrospray ionization (ESI) interface operating in the positive mode were employed. The separation was accomplished at 40 ◦C using a Zorbax Eclipse Plus C18 column (2.1 × 100 mm, 3.5 µm particle size) provided by Agilent (USA). The mobile phases for the column were LC-MS grade distilled water as solvent A (Merck Co.) and Acetonitrile gradient grade as solvent B (30:70, v/v) which contained formic acid (0.1%). The solvents flow rate was set at 1 mL/min. The injection volume was 5.0 µL in each run. Nitrogen (99.99%) was used as drying and collision gas.

The measurement of COD concentration, chloride ion, and oxygen level was according to the standard method. To measure the total organic carbon in water solutions, a TOC analyzer (model Shimadzu, Japan) was used according to standard methods. The intensity of UV radiation inside the reactor was measured and monitored by Hagner digital UV meter.

**2.4. Toxicity assessment**

To evaluate the final effluent's toxicity after performing the UV/S-ARP, ECOSAR toxicity evaluation software (ECOSAR, model v1.11) was used. US Environmental Protection Agency proposes this model as a useful model for assessing acute and chronic toxicity of various toxins. In recent years, this software was widely applied to evaluate the toxicity of pesticides. In this modeling approach, the contact time for fish and green algae is 96 hours and for Daphnia is 48 hours. Acute toxicity criteria ​​are EC50 for green algae and LC50 for fish and Daphnia. The output data of model for acute and chronic toxicity were evaluated based on UN GHS system, according to which, acute and chronic toxicity into four categories: 1. not harmful (LC50/EC50/chV>100), 2. harmful (100≥LC50/EC50/chV>10), 3. toxic (10≥LC50/EC50/chV>1), and 4. very toxic (LC50/EC50/chV≤1) (2011).

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## **3. Results and discussion**

**3.1 Degradation and kinetics of TCF by sulfite, UV and UV/S-ARP**

The efficiencies of three processes of UV alone, sulfite alone and UV/S-ARP were obtained and compared at initial pH of 7 within a reaction time of 60 min. The results are shown in Figs. 2 and 3. The results indicated that UV/S-ARP had a higher efficiency in TCF removal than the individual processes of UV and sulfite. The residual concentrations of TCF were 0.99, 0.31, and 0.04 mg/L for the sulfite, UV, and UV/S-ARP, respectively. Reduction efficiencies of 1, 69, and 96% were obtained for the sulfite, UV, and UV/S-ARP, respectively.

Since the addition of sulfite ions alone did not affect the reduction efficiency of TCF pesticides in the contaminated solution, the other two processes were evaluated and compared based on a pseudo-first-order reaction.

For a pseudo first-order reaction, the rates of reactions can be calculated by following equation:

(1)

In this equation, *t* is the contact time, and Kobs is the reaction rate constant that is calculated by the linear regression slope between the natural logarithm of [TCF] at time t and [TCF] at t0. The degradation of TCF is possible in two ways, one is photolysis by UV radiation, and the other is radical production, as displayed in the following equation:

Kobs= KUV+Kradical  (2)

In equation 2, KUV is the first-order reaction rate constant calculated for UV photolysis. According to Fig. 3, the values of Kobs for UV/S-ARP and UV photolysis were 0.0625 and 0.0184, respectively. Therefore, the Kradical value was calculated as 0.041 (min−1), which indicates that the role of produced radicals is much higher than that of photolysis alone by UV radiation. The presented result show that Kobs for UV/S-ARP was 3.4 times than UV alone, which was mostly accomplished by reducing radicals such as eaq −, H•, and SO3• − that were produced by UV/S-ARP (Entezari et al. 2019; Yu et al. 2019). Since the synergetic effect of UV photolysis by sulfite in UV/S-ARP is comparable due to radical production. However, the results show that the application of sulfite alone is not able to remove or degrade the TCF pesticide from aqueous solutions. This result was in agreement with the result obtained by Yu et al. (2020). In fact, sulfite ions do not have oxidizing or degrading properties for decomposing organic compounds. Using UV radiation has been able to degrade a significant amount of TCF pesticide in an aqueous solution. In this regard, some studies have shown that UV radiation can play an important performance in decomposing organic compounds and even resistant organic compounds. This could be due to TCF molecules' high absorption power of 253.7 nm (Yu et al. 2020). Due to the ability of UV/S-ARPS in the decomposition and removal of persistent organic compounds, the utilization of ARPs has been proposed as a new method for removing resistant contaminants from aquatic solutions (Liu et al. 2013; Trojanowicz et al. 2018; Yu et al. 2013; Yu et al. 2018; Yu et al. 2019; Yu et al. 2020). The results of this study on the ability to remove TCF as a toxic refractory organic compound in the aqueous environment are consistent with the results of other studies conducted by other researchers on the application of UV/S-ARP to remove resistant organics compounds such as 4,2-dichlorophenol, vinyl chloride, perfluoroctanate and diclofenac have been demonstrated using this method. The degradation ability of ARP for TCF pesticide can be due to the production of sulfite radicals, hydrated electrons, and hydrogen atoms. Similar studies have indicated the major role of hydrated electrons in the transformation and reduction of target pollutants with a reduction potential of -2.9 V. The use of this method for the removal of other halogenated organic compounds was reported. Due to the presence of chloride in the structure of TCF pesticide, the UV/S-ARP can play an effective performance in the decomposition of TCF through the dechlorination of the pesticide.

**3. 2. Effect of operational parameters**

**3.2.1 Effect of pH**

The results show that increasing the pH from 5 to 7 increases the process efficiency, but increasing the pH from 7 to 9 does not significantly improve the process performance. Therefore, the best pH for the UV/S-ARP was 7. The removal efficiency of TCF by the process was obtained 96% under neutral pH conditions. The hydrated radicals, sulfite radicals, and hydrogen ions play a major role in the ARP, and the rate of transformation in water depends on the type and amount of the abovementioned agents. The type and amount of above radicals are influenced by the pH of water solution; therefore, solution pH is very effective in the conversion of target pollutant by the water treatment process.

Increasing the pH to 7 can effectively increase the reduction process because UV absorbance spectra of the sulfite solutions are also pH-dependent and consequently increase the yield of radical species such as SO3• and eaq- radicals (Yu et al. 2018). Botlaguduru et al. showed that pH conditions greater than 7 favored the advanced reduction of the target pollutant that their results agreed with the results of our research. Other researchers indicated that the production of eaq- , H• and SO3• is pH-dependent. Moreover, Liu et al. report that sulfite ion (SO3-2) is the most dominant than other sulfite species such as bisulfite ion (HSO-3) and metabisulfite ion (S2O2-5) at neutral and alkaline pHs. So SO3-2 can absorb UV radiation and produce reactive radicals, but at lower pH, more bisulfite and metasulfite are produced, and the bisulfite ion absorbs a small amount of UV radiation and cannot produce active species.

**3.2.2 Effect of sulfite dosage**

Increasing the sulfite concentration from 60 to 120 mg/L increased the process efficiency from 80 to 96% (Fig. 5), but increasing sulfite concentration from 120 to 180 mg/L, a significant difference was not observed in the decomposition efficiency (2.4% increase in the efficiency). However, by increasing sulfite concentration from 120 to 180 mg/L, it might compete with TCF molecules for incident UV radiation inhibiting TCF photolysis and react with sulfite radicals. Hence, 120 mg/L of sulfite concentration was recommended in UV/S-ARP for the order to save sulfite consumption.

According to Beer-Lambert's law, increasing the sulfite concentration results in increasing the absorption of UV light in the solution, ultimately leads to an increase in the reduction efficiency of TCF (Nyangiwe et al. 2018). The results of this study regarding the effect of sulfite concentration are consistent with the results of study accomplished by Entezari et al. (2018) for other environmental pollutants (Entezari et al. 2019), Xie et al. (2017) and Jung et al. (2014) showed that increasing sulfite ion concentration caused to increased pollutants removal in ARPS because sulfite would absorb more UV radiation and originate more reductive radicals for decomposition of goal pollutants (Jung et al. 2014). Moreover, excessive increase of sulfite concentration can affect the direct photolysis process and reduce the effect (Xie et al., 2017).

**3.2.3 Effect of TCF concentration**

The rate of TCF reduction increased by increasing time from 15 to 60 min, and relatively complete reduction of TCF was obtained within 60 min (Fig. 6). As well as the effect of TCF concentration on the efficiency of the UV/S-ARP, reaction kinetics was investigated, and the results, including the values ​​of R2 ,were presented in Table 1. As it is obvious, the reaction rate coefficients decrease from 0.07 to 0.042 L/min with increasing TCF concentration from 2 to 10 mg/L, respectively. In pseudo-first-order kinetics, the degradation rate (robs) has a direct proportionality with the pollutant concentration. Therefore, as TCF concentration increases, the degradation rate is also higher. The higher degradation rate occurs for 10 mg/l concentration (robs=0.42 mg L-1 min-1). These results are reasonable since more TCF concentrations increase the probability of contact among the sulfite radicals, UV radiation, and TCF pesticide. Our results showed that the initial concentrations used can be removed at the desired time, i.e., 60 minutes of contact time with acceptable efficiency, although the removal rate is higher at lower concentrations, this process can degrade and remove the highest concentration of the test concentration, i.e., 10 mg/l, well from the water solution. In this regard, other studies can be performed to evaluate the ability of this process to remove higher concentrations, or economic studies can be studied to compare this process in the removal of different concentrations.

Our study shows that increasing the concentration of TCF results in a decrease in efficiency and kobs. These results are consistent with the study reported by Entezari et al. (2019). The reasons for the decrease in efficiency with increasing the concentration of TCF can be the lack of reaction time between reductive agent and TCF, lack of the reductive radicals for higher concentrations, and reduction of radical production due to adsorption of the high proportion of UV radiation by contaminant.

**3.2.4 Effect of UV radiation intensity**

For the radiation intensities of 540, 1091 and 1281 μW/cm2, the reduction efficiencies were obtained 74, 98.4 and 98.7, respectively (Fig. 7).

Considering there was no significant efficiency increase for 1091 and 1280 μW/cm2 radiation intensities, so 1091 μW/cm2 radiation intensity was selected as a suitable option both technically and economically (to save energy consumption). The removal efficiency increased by increasing the radiation intensity from 540 to 1091 μW/cm2. This phenomenon is explained by the fact that by increasing the intensity of UV light, the number of effective radicals produced is increased, and the amount of photolysis of the pollutant is increased as a result of the removal efficiency of TCF pesticide. These results are in agreement with the previously reported research for other organic pollutants in water solutions. According to studies conducted by others, the increase in the removal efficiency of pollutants with the increase of UV radiation intensity in the ARP may be due to increasing photolysis rate and increase of reductive radicals. By increasing the light intensity of UV from 1091 μW/cm2 to 1280 μW/cm2, a significant difference was not observed in the decomposition efficiency which can be due to the sufficient light intensity of the UV radiation to produce the radicals required for decomposition.

**3.3. Indirect identification of reducing agents**

Five scavengers, including oxygen, nitrite, nitrate, ammonium oxalate, and ethanol were used to study the mechanism of TCF reduction by the UV/S-ARP. The results were compared with the absence of scavengers, which are shown in Fig. 8. The results show that ethanol and ammonium oxalate has no significant effect on reduction efficiency, while oxygen, nitrite, and nitrate have the most effect on reduction efficiency.

Scavenging experiments with nitrite and nitrate scavengers (as eaq- and H• quencher) were accomplished to verify the performance of hydrated electrons and hydrogen radicals in the reduction of TCF pesticide. Eqs. 1 to 4 show that nitrite more quenches H• and this is due to the higher rate constant compared to nitrate (Eqs. 3 and 4), but due to the fact that acidic conditions are required to produce it, and this study was performed under neutral conditions, the probability of its effect on degradation is very low.

Sulfite radicals react rapidly with oxygen based on the Eq. 5 and SO5−• converted to SO4•− and SO42− through other chain steps.

As shown in Fig. 8, the addition of oxygen, nitrate, and nitrite reduces the efficiency of UV/S-ARP. The presence of oxygen as a sulfite radical quencher caused the most suppressive effect on reducing TCF by the UV/S-ARP compared with nitrate and nitrite ions as eaq− and H• quencher. However, oxygen can reduce the TCF removal efficiency by about 35%. The probability of the presence of H• as a reducing agent in our study is very low because my study was performed at neutral pH, but this radical is mostly formed at acidic pH (Botlaguduru et al. 2015). This indicates that radicals such as SO3o− and eaq- are responsible for the reduction and transformation of TCF, which is in agreement with the resulted report of Liu et al. for other contaminants.

**3.4. Reductive dechlorination in UV/S-ARP**

Fig. 9 shows the reduction efficiency of TCF pesticides versus the amount of chloride ions generated during the treatment process of UV/S. As illustrated in Fig. 9, as the efficiency of TCF removal increases, the amount of chloride ions released in the water solution increases.

Other researchers reported the reductive dichlorination via various ARPs. Therefore, increasing the concentration of chlorine ions released during the conversion of TCF indicated significant progress in the transformation of TCF. The removal of chlorine from the structure of TCF can lead to the formation of intermediates which can be more easily converted. However, another study showed that chloride ions are the main product of the advanced reduction of chlorine-containing organic compounds.

**3.5. Intermediate byproducts and reaction pathways of TCF reduction**

The intermediates generated during the transformation of TCF using ARP are shown in Table 2. The mass spectra for four intermediate products are shown in Fig. 10.

All of these compounds reach their maximum concentration in 30 min, which eventually reaches near zero over time (2 h). According to the identified degradation intermediates, the relevant reaction pathways were suggested in Fig. 11.

Based on LC-MS/MS analysis, TCF reduction leads to the production of several degradation intermediates, including (in ascending order): A) 2, 2-Dichlorovinyl methyl phosphate, B) dimethyl hydrogen phosphate, C) Demethyl trichlorfon, and D: methyl dihydrogen phosphate.

According to the results, the conversion of 1 to 2 and subsequently 3 is more probable than the other pathways. This result is in agreement with other ARPs studies performed for the removal of TCF.

**3.6. Mineralization of TCF by UV/S-ARP**

The results show that the reduction efficiencies based on TOC and COD tests in 60 min contact time using UV-sulfite process were 74.6±5.3 % and 79.5±4.9%, respectively.

The results of the TOC test and LC-MS/MS analysis (efficiencies 74.6 and 96%, respectively) indicate that the advanced UV-sulfite reduction process is not fully efficient for mineralization and conversion of final compounds. The results show that there is a direct relationship between mineralization and the degradation of TCF. According to the results, UV/S-ARP has the necessary efficiency and ability to break the C-Cl and C = C bands and can significantly reduce the toxin of TCF and the ability to use subsequent processes, including biological processes to decompose the intermediates produced by the reduction process.

**3.7. Bio-assessment of UV/S-ARP effluent**

The toxicity of TCF and dichlorvos as well as by-products of these compounds was evaluated using ECOSAR software of the US Environmental Protection Agency. The results are presented in Table 3. The values ​​of acute and chronic toxicities have been evaluated according to the system estimated by GHS (2011). According to this system, acute and chronic toxicities were classified into four categories according to the determined values. For not harmful materials, LC50/EC50/Chv values ​​greater than 100 are considered. For harmful, toxic, and very toxic substances, these values ​​are 100≥LC50/EC50/chV>10, 10≥LC50/EC50/chV>1, and LC50/EC50/chV≤1, respectively. The results of this study show that TCF pesticide and its intermediates have no acute toxicity to fish and daphnia (LC50/EC50/Chv higher than 100), but Demethyl trichlorfon have chronic and acute toxicity for alga. Moreover, 2, 2-Dichlorovinyl methyl phosphate has chronic toxicity for daphnia. But in general, in terms of acute toxicity, all intermediate products are in the category of not harmful substances and do not have significant acute effects on aquatic organisms. According to the toxicity test results, most by-products have less toxic effects on fish, daphnia, and green algae than TCF pesticide. By small amounts of the final product in this study, acute and chronic effects of TCF reduction are unlikely. According to the results, it can be concluded that using UV/S-ARP can eliminate the ecotoxicological effects of TCF in contaminated water. The results of this study show that the intermediate byproducts containing chlorine atoms have more acute and chronic effects on green algae and Daphnia than the other compounds. The results of this study are consistent with the results of Li et al. (2020).

In general, the results of this study showed that to fully ensure the ecotoxicological effects of effluent from the UV/S-ARP of TCF pesticide for aquatic organisms, it is suggested that further degradation of the effluent to ensure the adverse effects of some by-products should be done on daphnia and green algae, but with these conditions, it can be said with complete confidence that the by-products produced during the process do not have adverse effects on the fish.

**4. Conclusions**

This study's results showed that the UV/S-ARP can degrade TCF pesticides. Intermediate products are produced during the reduction of TCF pesticides which may require further treatment to remove these intermediates. Besides, the rate of reduction and removal of TCF and dichlorvos compounds using the UV/S-ARP was significant so that the amount remaining in the final solution was less than the recommended allowable values. The results of this study also show that in the degradation of TCF by UV/S-ARP, pH, initial concentration, reaction time, sulfite ion concentration, and UV intensity have played an important role in this process. Using this method, mineralization has not been done completely and the mineralization efficiency in the final effluent has been less than the degradation efficiency of the pesticide. Some degradation intermediates of TCF decomposition have more acute and chronic toxicity, but due to the very low concentration may not have adverse effects on the environmental sources. The results of the study show that the intermediate compounds obtained from the degradation have no adverse effects on fish. This process has the ability to remove chlorine from the TCF structure, thus improving the degradability of this pesticide by other processes. In general, the use of this process was able to remove more than 95% of TCF in water solution. The amount of mineralization based on removal of TOC and COD is 74.6% and 79.52%, respectively, which is an acceptable efficiency for reducing the amount of TCF in the solution contaminated with this pesticide.