Iğdır Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 13(1), 192-199, 2023
Journal of the Institute of Science and Technology, 13(1), 192-199, 2023

ISSN: 2146-0574, eISSN: 2536-4618

Environmental Engineering **DOI: 10.21597/jist.1157037**

Research Article

Received: 05.08.2022 **Accepted: 31.10.2022**

To Cite: Çelik, M., Aydın, E., Çelik, A. & Gürtekin, E. (2023). Degradation of Oxytetracycline and Chlortetracycline by Fenton Process. *Journal of the Institute of Science and Technology*, 13(1), 192-199.

Degradation of Oxytetracycline and Chlortetracycline by Fenton Process

Murat ÇELİK[1], Ekrem AYDIN[1], Aytekin ÇELİK[1], Engin GÜRTEKİN[1*]

Highlights:

- Oxytetracycline and chlortetracycline was degraded with Fenton process.
- The operating conditions of the Fenton process have been optimized.
- The Fenton process can be used for oxytetracycline and chlortetracycline degradation

Keywords:

- Degradation
- Fenton process
- Oxytetracycline
- Chlortetracycline

ABSTRACT:

In this study, degradation of oxytetracycline (OTC) and chlortetracycline (CTC) was investigated by Fenton process. In experimental studies conducted for this purpose, different values of pH, Fe^{2+} concentration, H_2O_2 concentration and reaction time were tested as important parameters for the Fenton process and optimum conditions for OTC and CTC removal were determined. For both antibiotics, the optimum values of the parameters in the Fenton process were the same, and these values were found to be 3 for pH, 20 mg/L for Fe^{2+} concentration, 25 mg/L for H_2O_2 concentration, 5 min for reaction time. Under these optimum conditions, OTC degradation was 100%, CTC degradation was 99.68%, chemical oxygen demand (COD) removal efficiency for oxytetracycline (O-COD) was 89.6%, COD removal efficiency for chlortetracycline (C-COD) was 88.5%. These results show that the Fenton process is an effective method that can be used for OTC and CTC degradation.

[1] Murat ÇELİK (**Orcid ID:** 0000-0002-1038-3450), Ekrem AYDIN (**Orcid ID:** 0000-0003-1985-7701), Aytekin ÇELİK (**Orcid ID:** 0000-0003-4234-1797), Engin GÜRTEKİN (**Orcid ID:** 0000-0003-3075-7891), Fırat University, Faculty of Engineering, Department of Environmental Engineering, Elazıg, Turkey

***Corresponding Author:** Engin GÜRTEKİN, e-mail: egurtekin@firat.edu.tr

INTRODUCTION

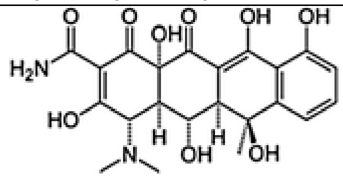
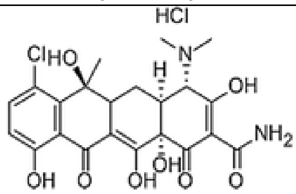
Tetracycline group antibiotics are widely used in human and animal treatment in the world due to their low cost and high efficiency (Çelik et al., 2018; Wang et al., 2018). Oxytetracycline and chlortetracycline are two of the most commonly used tetracycline group antibiotics. The main chemical structure and physicochemical properties of oxytetracycline and chlortetracycline are given in Table 1. Oxytetracycline and chlortetracycline with their low adsorption and metabolizability are transported to the environment with urea and feces. Therefore, they are present even in low concentrations in environments such as surface water, soil, groundwater, seawater, sediment (Liu et al., 2016). These antibiotics pose a potential risk to human health and ecosystem through the development of bacteria and pathogens that resist even at low concentrations (Alalm et al., 2015; Aydın et al., 2016). For this reason, antibiotics need to be removed before moving to environmental mediums. Biological treatment methods are insufficient in antibiotic removal due to their resistant and toxic structures. Advanced oxidation methods are widely used in the treatment of wastewater containing recalcitrant and non-biodegradable organic compounds (Cahino et al., 2018). Advanced oxidation methods are based on the formation of hydroxyl radicals and can oxidize a wide range of organic matter to CO_2 and H_2O (Sun et al., 2009). Methods such as Fenton, photo-Fenton, ozonation, electrochemical oxidation and photocatalysis are the most commonly used advanced oxidation processes (Mousavi et al., 2018). In the Fenton process, these recalcitrant and non-biodegradable complex organic compounds are oxidized with the •OH formed by Fe^{2+} and H_2O_2 reaction (Eqs.1-2) (Hakika et al., 2019). Fenton process is one of the advanced oxidation methods with its advantages such as high oxidation power, fast oxidation kinetics, relatively low cost and ease of operation and maintenance (Gürtekin and Sekerdağ, 2008; Sun et al., 2009).

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

$$\text{Organics} + \bullet OH \rightarrow \text{products} \quad (2)$$

Oxytetracycline and chlortetracycline are recalcitrant and non-biodegradable micropollutants. The aim of this study is to investigate the degradation of oxytetracycline and chlortetracycline with Fenton process. For this purpose, optimization values of factors such as pH, Fe^{2+} concentration, H_2O_2 concentration and reaction time as important parameters for Fenton process were determined.

Table 1. Chemical structure and physicochemical properties of the OTC and CTC (Daghrir and Drogui, 2013)

Compound	**Oxytetracycline hydrochloride**	**Chlortetracycline hydrochloride**
Structure		
Molecular formula	$C_{22}H_{24}N_2O_9$. HCl	$C_{22}H_{23}ClN_2O_8$. HCl
Molecular weight	496.9 g/mol	515.3 g/mol
Water solubility	0.062 mol/L	0.008 mol/L
pKa	3.2, 7.46, 8.9	3.3, 7.55, 9.3
Usage	Antibiotic	Antibiotic

MATERIALS AND METHODS

Experimental System

All experiments were carried out in amber colored glass bottles with a total volume of 250 mL to prevent possible photodegradation by light exposure. Reactions were provided with agitators adjusted at 25 °C. The pH was initially adjusted with the help of H_2SO_4 and NaOH using a Thermo Scientec brand pH meter and no other pH adjustment was made during the reaction. For each experiment, a certain amout of Fe^{2+} and H_2O_2 was added to the oxytetracycline and chlortetracycline solutions prepared in different reaction vessels. At the end of the reaction, the pH was adjusted to 9-10 levels to allow precipitation and subjected to precipitation for 90 minutes. At the end of the precipitation time, clear liquid was taken from the upper part of the reactor and OTC, CTC and COD were analyzed.

Reagents

Oxytetracycline (OTC) hydrochloride salt and Chlortetracycline (CTC) hydrochloride salt were purchased from Sigma-Aldrich and 35% purity Hydrogen peroxide (H_2O_2) was purchased from Merck. Iron sulfate heptahydrate was obtained from Biochem. The pH of the solution was initially adjusted with dilute 98% sulfuric acid (0.2 and 0.02 N H_2SO_4) and sodium hydroxide (0.2 N NaOH). OTC and CTC solutions were prepared by diluting the OTC and CTC stock solution (200 mg/L). All solutions were prepared with deionized deoxygenated water (DDW) and stored at 4 °C.

Analytical methods

OTC, CTC and COD analyzes were performed on the samples taken at the end of the precipitation period in order to determine the performance of Fenton process. OTC and CTC determination was performed with Shimadzu brand High Performance Liquid Chromatography (HPLC) device. As mobile phase, a mixture of 20 mM (NH_4) H_2PO_4 and HPLC purity acetonitrile with pH adjusted between 2.45-2.55 was used, and the flow rate was set at 1.2 mL/min and the injection volume was set at 100 μL. OTC and CTC analyzes were detected by a diode array detector at a wavelength of 269 nm and a retention time of 2.95 and 7.95 mins, respectively. Calibration was done with five standards between 20 and 100 mg/L and R^2 was higher than 0.998. COD determination was made with Merck brand 114895 coded kit.

RESULTS AND DISCUSSION

Effect of pH

pH is an important parameter in the Fenton process as it affects hydroxyl radical formation and the concentration of Fe forms. The pH range in which the optimum hydroxyl radical is formed in Fenton process is 2 to 4 (Verma and Haritash, 2019). Based on the literature, pH 2, 3 and 4 values were used in this study. Studies were carried out to determine the effect of pH by using OTC and CTC concentration of 100 mg/L, Fe^{2+} concentration of 30 mg/L and H_2O_2 concentration of 300 mg/L. At pH 1 and 2, the degradation efficiency decreases because hydroxyl radicals are scavenged by the H^+ ion. Hydrogen peroxide below pH 2 can possibly remain solvated to form an oxonium ion, which improves its stability and significantly reduces its reaction with iron ions (Sun et al., 2009). Complex species such as $[Fe(H_2O)_6]^{2+}$ and $[Fe(H_2O)_6]^{3+}$ also react more slowly with hydrogen peroxide. At values higher than pH 4, degradation efficiency decreases with the precipitation of iron ion and reduction of decomposition of hydrogen peroxide to hydroxyl radical (Vasseghian et al., 2022). Oxytetracycline and chlortetracycline are also available in different forms depending on pH. Oxytetracycline has pKa values of 3.57, 7.49 and 9.88. $OTCH_3^+$ form at pH values less than 3.57, $OTCH_2^{\pm}$ form in the range 3.57-7.49, $OTCH^-$ form in the range of 7.49-9.88, and OTC^{2-} form in the values greater than 9.88 are the dominant form (Liu et al., 2015). Chlortetracycline has pKa values of 3.30, 7.44 and 9.27. CTH_3^+ form with pH value less than 3.3, $CTH_2^{\pm}$ form in the range of 3.30-7.44, CTH^- form in the range of 7.44-9.27 and CTC^{2-} form with values greater than 9.27 are the dominant form (Liu et al., 2018). The effluent OTC values were given in Figure 1 and the effluent OTC values at pH 2, 3 and 4 were found to be 1.729, 0.189 and 2.99 mg/L, respectively. The effluent COD concentration for oxytetracycline (O-ECOD) was obtained as 4, 3.5 and 5.9 mg/L for pH 2, 3 and 4, respectively. Based on the results of this experimental study, the optimum pH value for oxytetracycline was found to be 3 and this pH value was used in the rest of the study. The effluent CTC concentration was found to be zero for pH 2, 3 and 4. The effluent COD concentration for chlortetracycline (C-ECOD) at pH 2, 3 and 4 was found to be 15, 15 and 24 mg/L, respectively. In addition, the size of the complexes formed is taken into account (data not given). When the effluent values and the size of the complexes formed were evaluated, the optimum pH value for CTC was found to be 3. OTC's main biodegradation products are 4-epi-OTC (EOTC), 2-acetyl-2-decarboxy-amido-OTC (ADOTC), α-apo-OTC and β-apo-OTC (Li et al.,2019). The transformation products of CTC have been reported to be 4-epichlorotetracycline (ECTC), anhydrochlorotetracycline (ACTC), and 4-epi-anhydrochlortetracycline (EACTC) (Aydın et al., 2016). In the study investigating the oxidation of sulfadiazine by the Fenton process, the optimum pH was found to be 3 (Yang et al., 2014). In another study, in which wastewater containing ibuprofen was purified by the Fenton process, the optimum pH value was determined as 3.5 (Odabasi and Buyukgungor, 2017). It is known that the protonated forms of oxytetracycline and chlortetracycline have little interest in the hydroxyl radical (Han et al., 2020). Despite the protonated form of oxytetracycline and chlortetracycline at this optimum pH, it has been observed that the hydroxyl radical formed in the Fenton process was effective.

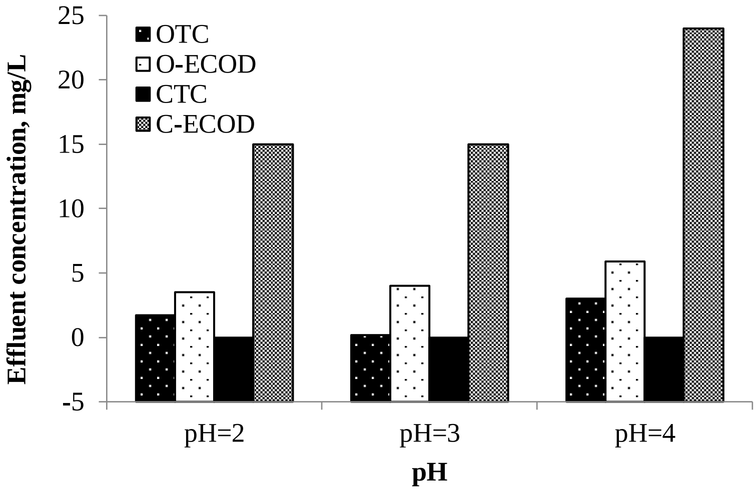


Figure 1. Effluent OTC, CTC, O-ECOD and C-ECOD concentrations at different pH values (OTC: 100 mg/L, CTC: 100 mg/L, Fe^{2+}: 30 mg/L, H_2O_2: 300 mg/L)

Effect of Fe^{2+} Concentration

This stage of the experimental study was carried out at concentrations of 0, 10, 20 and 40 mg/L Fe^{2+}. The initial OTC and CTC concentration was 100 mg/L, the selected H_2O_2 concentration was 300 mg/L and the pH value was 3. The effects of different Fe^{2+} concentrations on OTC and CTC removal were determined (Figure 2). Effluent OTC values obtained at 0, 10, 20 and 40 mg/L Fe^{2+} concentration were found to be 2.57, 1.12, 0 and 0 mg/L, respectively, while effluent CTC concentration values were 3.6, 0, 0 and 0.12 mg/L, respectively. The O-ECOD values at 0, 10, 20 and 40 mg L Fe^{2+} concentrations were 13, 12, 5 and 8 mg/L, and the C-ECOD values were 140, 36, 16 and 24 mg/L, respectively. From both the effluent OTC, CTC and O-ECOD, C-ECOD values, the optimum Fe^{2+} concentration value for OTC and CTC removal appears to be 20 mg/L. Increasing the Fe^{2+} concentration up to 20 mg/L increased the •OH and concentrations greater than 20 mg/L caused a decrease in OTC and CTC removal and an increase in effluent COD values due to the self-scavenging effect of the •OH. In a study conducted with the Fenton process, it was stated that increasing iron concentration had a negative effect on color removal (Kaya and Ascı, 2019). In the oxidation of norfloxacin by Fenton process, 100% removal efficiency was obtained at 5 mg L Fe^{2+} concentration (Cruz et al., 2012).

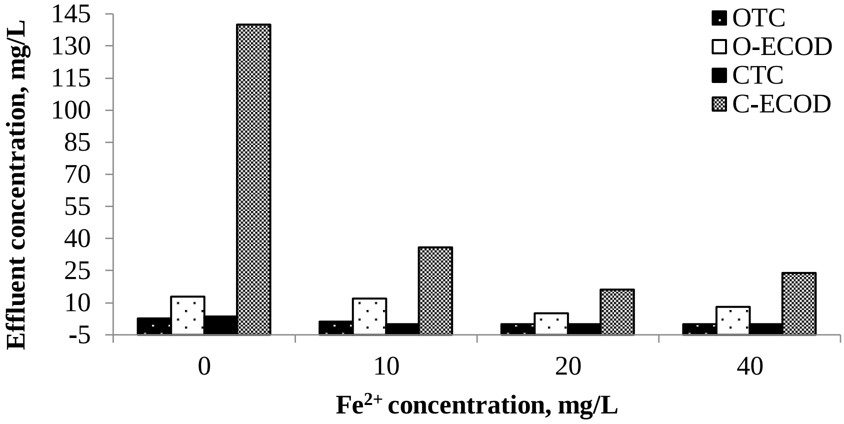


Figure 2. Effluent OTC, CTC, O-ECOD and C-ECOD concentrations at different Fe^{2+} values (OTC: 100 mg/L, CTC: 100 mg/L, pH: 3, H_2O_2: 300 mg/L)

Effect of H_2O_2 Concentration

Effluent OTC, CTC, O-ECOD and C-ECOD concentrations at different H_2O_2 concentrations are given in Figure 3. It was seen that there was no decrease in the effluent values of OTC, CTC, O-ECOD and C-ECOD with the increase of H_2O_2 concentration. The optimum concentration value for H_2O_2 is 25 mg/L under the initial OTC and CTC concentration of 100 mg/L, Fe^{2+} concentration of 20 mg/L and pH 3 operating conditions. It has been stated that the decrease in the rate of degradation at higher H_2O_2 concentrations is due to both the recombination of hydroxyl radicals and the scavenging effect of the •OH of H_2O_2 (Dalgic et al., 2017). However, such a critical H_2O_2 concentration value was not found in this study. At the same time, excessive use of H_2O_2 is not recommended as residual H_2O_2 concentration contributes to COD. In a similar study, 100 mg/L was found to be the optimum value for H_2O_2 concentration by looking at the efficiency of tetracycline and COD removal (Gürtekin et al., 2022). In the oxidation of carbofuran by Fenton process, 81% of carbofuran was degraded at a concentration of 100 mg/L H_2O_2 (Ma et al., 2010).

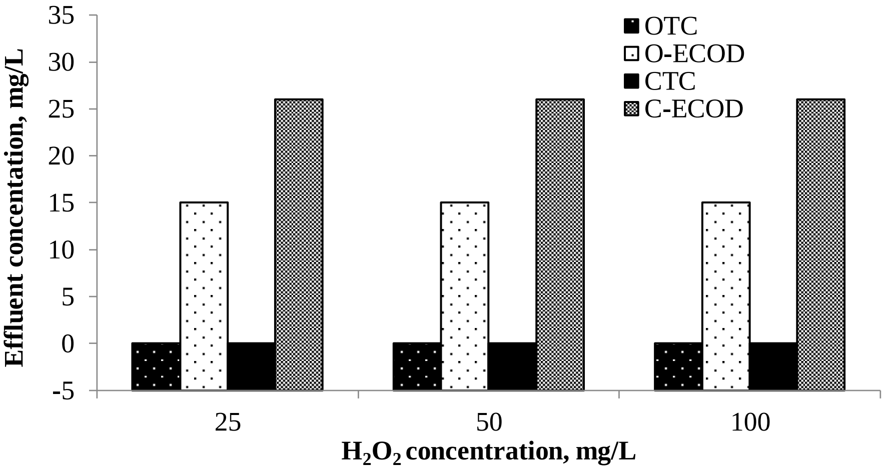


Figure 3. Effluent OTC, CTC, O-ECOD and C-ECOD concentrations at different H_2O_2 values (OTC: 100 mg/L, CTC: 100 mg/L, pH: 3, Fe^{2+}: 20 mg/L)

Effect of Reaction Time

Figure 4 shows the effluent OTC, CTC, O-ECOD and C-ECOD concentrations obtained at different reaction times. While the increase of reaction time increases degradation efficiency, it increases the operating cost. When these two conditions are evaluated together, it is seen that the optimum reaction time is 5 min for both antibiotic removal. Elmolla and Chaudri (2009) studied the treatment of amoxycillin, ampicillin and cloxacillin antibiotics at the initial concentration of 104, 105 and 103 mg/L, respectively, and stated that complete disintegration of these antibiotics was achieved within 2 min of reaction time under optimum conditions. It was stated that a reaction time of 10-15 minutes was sufficient for near maximum biodegradability in the removal of chlortetracycline by photocatalytic ozonation processes. (Hammad Khan et al., 2013) In another study investigating the degradation of captopril by the Fenton process, 95% of captopril degradation was obtained in a reaction time of 15 minutes (Lima Santos et al., 2021).

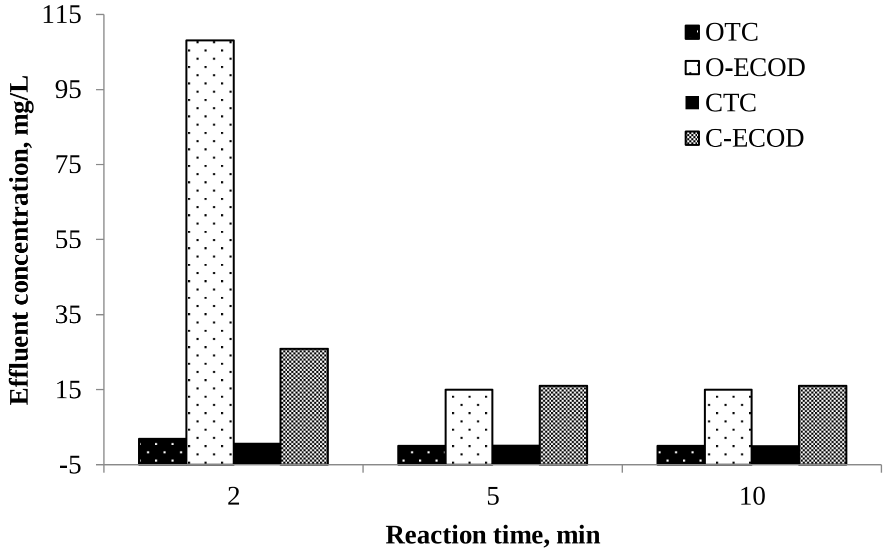


Figure 4. Effluent OTC, CTC, O-ECOD and C-ECOD concentrations at different reaction time (OTC: 100 mg/L, CTC: 100 mg/L, pH: 3, Fe^{2+}: 20 mg/L, H_2O_2: 25 mg/L)

CONCLUSION

In the Fenton process, the main factors such as pH, Fe^{2+} concentration, H_2O_2 concentration and reaction time are important operating parameters and systematic study is necessary for optimization. Optimum conditions in Fenton process were the same for OTC and CTC. These conditions were determined as 3 for pH, 20 mg/L for Fe^{2+} concentration, 25 mg/L for H_2O_2 concentration and 5 min for reaction time. Under these optimum conditions, OTC degradation and O-COD were 100% and 89.6%, respectively. CTC degradation and C-COD were 99.68% and 88.5%, respectively. These results showed that OTC and CTC were fully degraded. When the COD parameter was evaluated, it means that approximately 90% of OTC and CTC were degraded to CO_2 and H_2O, and about 10% of them to different intermediates. It was determined that Fenton process is a method that can be used for the degradation of OTC and CTC, which are among the tetracycline group.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

REFERENCES

Alalm, M. G., Tawfik, A. & Ookawara, S. (2015). Degradation of four pharmaceuticals by solar photo-Fenton process: kinetics and costs estimation. *Journal of Environmental Chemical Engineering*, 3(1), 46-51.

Aydın, E., Şahin, M., Taşkan, E., Hasar, H. & Erdem, M. (2016). Chlortetracycline removal by using hydrogen based membrane biofilm reactor. *Journal of Hazardous Materials*, 320, 88-95.

Cahino, A. M., de Andrade, M. M. A., de Araújo, E. S., Silva, E. L., Cunha, C. D. O. & Rocha, E. M. R. (2018). Degradation of tetracycline by solar photo-Fenton: Optimization and application in pilot photoreactor. *Environmental Quality Management*, 28(1), 101-106.

Çelik, A., Casey, E. ve Hasar, H. (2018). Degradation of oxytetracycline under autotrophic nitrifying conditions in a membrane aerated biofilm reactor and community fingerprinting. *Journal of Hazardous Materials*, 356, 26-33.

Daghrir, R. & Drogui, P. (2013). Tetracycline antibiotics in the environment: a review. *Environmental Chemistry Letter*, 11(3), 209-227.

Dalgic, G., Turkdogan, I. F., Yetilmezsoy, K. & Kocak, E. (2017). Treatment of real paracetamol wastewater by Fenton process. *Chemical Industry and Chemical Engineering Quarterly*, 23(2), 177-186.

De la Cruz, N., Giménez, J., Esplugas, S., Grandjean, D., De Alencastro, L. F. & Pulgarin, C. (2012). Degradation of 32 emergent contaminants by UV and neutral photo-fenton in domestic wastewater effluent previously treated by activated sludge. *Water Research*, 46(6), 1947-1957.

Elmolla, E. S. & Chaudhuri, M. (2009). Degradation of the antibiotics amoxicillin, ampicillin and cloxacillin in aqueous solution by the photo-Fenton process. *Journal of Hazardous Materials*, 172, 1476-1481.

Gürtekin, E. ve Şekerdağ, N. (2008). An advanced oxidation process: Fenton process. *Pamukkale University Journal of Engineering Sciences*, 14(3), 229-236.

Gürtekin,, E., Çelik, M., Aydın, E. ve Çelik, A. (2022). Degradation and mineralization of tetracycline by Fenton process. *Environmental Research and Technology*, *5*(2), 181-187.

Hakika, D. C., Sarto, S., Mindaryani, A. & Hidayat, M. (2019). Decreasing COD in sugarcane vinasse using the fenton reaction: The effect of processing parameters. *Catalysts*, 9(11), 881.

Hammad Khan, M., Jung, H. S., Lee, W. & Jung, J. Y. (2013). Chlortetracycline degradation by photocatalytic ozonation in the aqueous phase: mineralization and the effects on biodegradability. *Environmental Technology*, 34(4), 495-502.

Han, C. H., Park, H. D., Kim, S. B., Yargeau, V., Choi, J. W., Lee, S. H. & Park, J. A. (2020). Oxidation of tetracycline and oxytetracycline for the photo-Fenton process: Their transformation products and toxicity assessment. *Water Research*, 172, 115514.

Li, Z. J., Qi, W. N., Feng, Y., Liu, Y. W., Ebrahim, S. & Long, J. (2019). Degradation mechanisms of oxytetracycline in the environment. *Journal of Integrative Agriculture*, 18(9), 1953-1960.

Lima Santos, L. R., de Souza Moreira, C. P., Quinino Dutra, R. C., de Sousa Ribeiro Vasconcelos, O. M., Magalhães Starling, S. M. & Gomes Mol, M. P. (2021). Degradation of the pharmaceutical captopril via Fenton process. *Environmental Engineering and Management Journal*, 20(11), 1739-1744.

Liu, Y., He, X., Duan, X., Fu, Y. & Dionysiou, D. D. (2015). Photochemical degradation of oxytetracycline: influence of pH and role of carbonate radical. *Chemical Engineering Journal*, 276, 113-121.

Liu, Y., He, X., Duan, X., Fu, Y., Fatta-Kassinos, D. & Dionysiou, D. D. (2016). Significant role of UV and carbonate radical on the degradation of oxytetracycline in UV-AOPs: kinetics and mechanism. *Water Research*, 95, 195-204.

Liu, Y., Wang, C., Sui, Z. & Zou, D. (2018). Degradation of chlortetracycline using nano micro-electrolysis materials with loading copper. *Separation and Purification Technology*, 203, 29-35.

Ma, Y. S., Sung, C. F. & Lin, J. G. (2010). Degradation of carbofuran in aqueous solution by ultrasound and Fenton processes: effect of system parameters and kinetic study. *Journal of Hazardous Materials*, 178(1-3), 320-325.

Mousavi, S. A., Farrokhi, F., Kianirad, N. & Falahi, F. (2018). Degradation of aniline from aqueous solution by Fenton process: modeling and optimization. *Desalination and Water Treatment*, 125, 68-74.

Odabasi, S. U. ve Buyukgungor, H. (2017). Investigation and evaluation of degradability of ibuprofen from wastewater by using fenton process. *European Journal of Engineering and Natural Sciences*, 2(1), 114-119.

Şefika,, K. ve Yeliz, A. (2019). Evaluation of color and COD removal by Fenton and photo-Fenton processes from industrial paper wastewater. *Journal of the Institute of Science and Technology*, 9(3), 1539-1550.

Sun, S. P., Guo, H. Q., Ke, Q., Sun, J. H., Shi, S. H., Zhang, M. & Zhou, Q. (2009). Degradation of antibiotic ciprofloxacin hydrochloride by photo-Fenton oxidation process. *Environmental Engineering Science*, 26(4), 753-759.

Vasseghian, Y., Almomani, F., Moradi, M. & Dragoi, E. N. (2022). Decontamination of toxic Malathion pesticide in aqueous solutions by Fenton-based processes: Degradation pathway, toxicity assessment and health risk assessment. *Journal of Hazardous Materials*, 423, 127016.

Verma, M. & Haritash, A. K. (2019). Degradation of amoxicillin by Fenton and Fenton-integrated hybrid oxidation processes. *Journal of Environmental Chemical Engineering*, 7(1), 102886.

Wang, J., Zhou, B., Ge, R., Song, T. S., Yu, J. & Xie, J. (2018). Degradation characterization and pathway analysis of chlortetracycline and oxytetracycline in a microbial fuel cell. *RSC Advances*, 8, 28613-28624.

Yang, J.F., Zhou, S. B., Xiao, A. G., Li, W. J. & Ying, G. G. (2014). Chemical oxidation of sulfadiazine by the Fenton process: kinetics, pathways, toxicity evaluation. *Journal of Environmental Science and Health, Part B*, 49(12), 909-916.