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Treatment of food industry wastewater by •OH-based electrochemical-Fenton method and toxicity evaluation

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ABSTRACT

This study investigated the treatment of food industry wastewater producing vinegar •OH-based electrochemical-Fenton (EF) processes using a sacrificial Fe anode in the presence of hydrogen peroxide (H_2O_2). The influence of current density, electrolyte concentration and H_2O_2 concentration on •OH-based EF treatment mechanism, kinetics and toxicity was investigated. Response surface methodology (RSM) was used for the optimization of these EF treatment processes parameters and the genotoxicity of treated water was evaluated with the removal performance after treatment. •OH-based EF processes achieved consistently yielding 99% chemical oxygen demand (COD) with a current density of 20 mA/cm^2 with the addition of sodium sulfate (Na_2SO_4) as the supporting electrolyte at a concentration of 5 and 60 mM H_2O_2 consuming 39.375 kWh/kg energy. RSM study showed that the current density was the most effective parameter on •OH-based EF processes via determining role in in-situ production of both Fe^{2+} and H_2O_2. The β-galactose induction ratios indicating the occurrence of DNA damage of *Salmonella typhimurium* TA1535/pSK1002 bacteria exposed to treated and untreated wastewaters were identified in the umuC AQ genotoxicity test, and they were reduced below 1.5 that is the specified limit value for genotoxicity. This study showed that non-biodegradable and toxic vinegar industry wastewater was successfully treated with •OH-based EF reducing genotoxicity even if it had a high COD value, the first time in the literature.

Keywords: Food industry wastewater; •OH-based electrochemical-Fenton; Response surface methodology; Chemical oxygen demand; umuC

1. Introduction

Food industry wastewater with high organic loads cannot be treated by classical methods because it is not possible to achieve sufficient levels of removal efficiency. Wastewater production of the food industry is quite special, ranging according to the type and production method of the product, the treatment methods also vary accordingly. Unfortunately, it remains a challenge to efficiently and accurately treat food wastewater due to its relatively high level of acidity and biological contamination level [1]. Due to its diverse effects on microorganisms, wastewater produced by vinegar-producing processes belongs in this category [2].

Vinegar is defined as the product by fermenting sugar-containing fruits to produce ethyl alcohol, followed by fermentation to acetic acid. Therefore, vinegar production wastewater significantly contributes to the overall food-related wastewater disposal, in terms of volume and organic contamination. The wastewater related to the production of vinegar in the food industry is very acidic [3] and most microorganisms may not bear this acidity, making some popular biological treatment methods obsolete [4]. Consequently, it is stated that the classical treatment of

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vinegar wastewater is difficult, and the treatment levels are rather low in the literature, it is argued that Fenton, electrochemical (EC) treatments and their hybrid application like electrochemical-Fenton (EF) may be a reasonable method especially non-biodegradable, toxic and soluble organic pollutant can be found in food industry wastewater [5]. It was shown that the Fenton process applied to biologically non-degradable toxic wastewater produces less secondary pollution. In another survey studied by Babuponnusami and Muthukumar [6] about sono-Fenton, photo-Fenton and electrochemical-Fenton (EF), it was observed that these methods successfully remove toxic contamination, which could not be totally treated by other conventional methods.

The Fenton (F) process depends on the reaction of ferrous ions (Fe^{2+}) with hydrogen peroxide (H_2O_2) under acidic conditions as homogenous advanced oxidation processes (AOPs). Continuous production of hydrogen peroxide and catalytic reduction of Fe^{3+} (to yield Fe^{2+} ions) causes increased hydroxyl production. The decomposition of H_2O_2 begins with an iron ion, which is then catalyzed and hydroxyl radicals (OH^-) are formed. Hydroxyl radicals (OH^-) react with all organic substances resulting in the formation of carbon dioxide (CO_2) and hydrogen peroxide H_2O_2 as the final product, thus organic materials are removed from water and wastewater. The reactions that occurred in water are given in Eqs. (1)–(6) [7].

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

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

$$Fe^{3+} + H_2O_2 \leftrightarrow Fe^{-}OOH^{2+} + H^+ \quad (3)$$

$$Fe^{-}OOH^{2+} \rightarrow HO_2^{\bullet} + Fe^{2+} \quad (4)$$

$$Fe^{2+} + HO_2^{\bullet} \rightarrow Fe^{2+} + O_2 + H^+ \quad (5)$$

$$OH^- + H_2O_2 \rightarrow H_2O + HO_2 \quad (6)$$

Factors affecting the removal efficiency of the Fenton process are Fe^{2+}, Fe^{3+}, H_2O_2 concentrations, pH, temperature and amount of organic and inorganic pollutants. With the Fenton process, toxic aliphatic and aromatic compounds such as phenol and derivatives, explosives, aniline, carbon tetrachloride can be removed from water with high removal efficiency. The hydroxyl radicals formed by the Fenton process oxidize organic materials to form reactive and oxidizable organic radicals. Fenton (F) is an effective process for the removal of color and chemical oxygen demand (COD) caused by different dyes such as reactive, direct, basic, acid and disperse dyes. In addition to these advantages, the release of additional Fe^{2+}, Fe^{3+} and H_2O_2 chemicals into the water is a limiting factor in the use of this method.

In EC treatment, the following two types of mechanisms are formed during the use of iron electrodes in wastewater expressed as Eqs. (7)–(14) [8].

First mechanism:
Anode:

$$4Fe_{(s)} \rightarrow 4Fe^{+2} + 8e^- \quad (7)$$

$$4Fe^{+2} \rightarrow 10H_2O + O_{2(g)} \rightarrow 4Fe(OH)_3 + 8H^+ \quad (8)$$

Cathode:

$$8H^+ + 8e^- \rightarrow 4H \quad (9)$$

Overall reaction:

$$4Fe_{(s)} + 10H_2O + O_2 \rightarrow 4Fe(OH)_3 + 4H_{2(g)} \quad (10)$$

Second mechanism:
Anode:

$$4Fe_{(s)} \rightarrow 4Fe^{+2} + 8e^- \quad (11)$$

$$Fe^{+2} + 2OH^- \rightarrow Fe(OH)_2 \quad (12)$$

Cathode:

$$2H_2O + 2e^- \rightarrow H_{2(g)} + 2OH^- \quad (13)$$

Overall reaction:

$$Fe_{(s)} + 2H_2O \rightarrow Fe(OH)_2 + H_{2(g)} \quad (14)$$

In EC treatment, the inorganic and undissolved organic pollutants in the water are removed by clinging to the formed $Fe(OH)_2$ and $Fe(OH)_3$ and producing easily settleable flocs. When it comes to removing the molecularly dissolved organic pollutants from wastewater, EF treatment is frequently examined in the literature. In the EF method, Fe^{2+} and H_2O_2 are produced electrochemically in situ instated of chemical addition from outside to remove molecularly dissolved biologically difficult contaminants (i.e., aromatic), toxic [9] and pharmastatic compounds, dying chemicals [10] gives promising results as compared to other physical, mechanical and biological treatment methods [11]. In general usage, the electrochemically formed Fe^{2+} ions are activated common oxidants addition from outside, such as hydrogen peroxide (H_2O_2), peroxymonosulfate (PMS), and peroxodisulphate (PS) to produce reactive free radicals (${}^{\bullet}OH$ and SO_4^{-}) because the in-situ production of these radicals required high current density application and longer hydraulic retention time resulted in high energy consumption. H_2O_2 activation and PMS or PS activation by electrochemically produced Fe^{2+} are generally referred to as hydroxyl based electrochemical-Fenton (${}^{\bullet}OH$-based EF) and electrochemical-Fenton-like (EF-like) processes, respectively [12]. The H_2O_2 is efficiently activated by electrochemically generated Fe^{2+}/Fe^{3+} ions, generating the reactive oxidative species which could accelerate the organic degradation process according to Eqs. (15)–(21) in ${}^{\bullet}OH$-based EF [13].

EC process:
At the anode:

$$Fe \rightarrow Fe^{2+} + 2e^- \quad (15)$$

At the cathode:

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \quad (16)$$

$$O_{2(g)} + 2H^+ + 2e^- \rightarrow H_2O_2 \quad (17)$$

EF process:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^\bullet OH + OH^- \quad (18)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{3+} + HO_2^\bullet + H^+ \quad (19)$$

$$H_2O_2 + e^- \rightarrow {}^\bullet OH + OH^- \quad (20)$$

$$^\bullet OH + \text{organics} \rightarrow \text{intermediates} + CO_2 + H_2O \quad (21)$$

In particular, $^\bullet$OH-based EF processes were shown to be efficient electrochemical methods for mineralizing a variety of dissolved organic pollutants such as pharmaceutical micropollutants, pesticides, and organic pollutants [14]. The toxicities of the intermediates produced from dissolved organic compounds found in the food industry wastewater during treatment must be investigated because the intermediates products of these pollutants can be more toxic than influent. In a work by Ansari and Malik [15], it was observed that genotoxicity of treated vinegar wastewater using Ames essay was necessary prior to utilization of treated wastewater in agricultural watering, due to its eminent toxicity. In another work by Arienzo et al. [16], it was concluded that toxicity analysis of treated vinegar wastewater is critical prior to disposal in wetlands. Similarly, Ioannou and Fatta-Kassinos [17] observed that vinegar wastewater treatment by sono-photo-Fenton reduces COD by 70%, dissolved organic carbon by 53% and color by 75%, but they reported that the product water still carried toxic effects. Yet, treatment results have not been reported for vinegar wastewater using EF, in the literature. In highly efficient advanced oxidation processes such as EF, there is a risk of forming more toxic components if the removal is not completed while oxidizing the pollutant carbon dioxide and water. Therefore, toxicity assessment should definitely be done in AOP processes such as EF.

In this study, the food industry wastewater producing vinegar, which has acidic characteristics and carries a high contaminant load, was treated by electrochemical-Fenton (EF) method. Treatment levels were obtained together with the necessary toxicity analysis. According to the achieved results, a discussion was presented regarding where and under which circumstances treated food industry wastewater could be used giving response surface methodology (RSM) analysis of EF method with sludge characterization.

2. Material and methods

This work introduced the $^\bullet$OH-based EF method for electrochemical treatment of real food industry wastewater by means of optimizing its working conditions and parameters, and by analyzing the resultant toxicity. The methods consist of $^\bullet$OH-based EF assays with various parameters and genotoxicity analysis of the treated wastewater. For the characterization of sludge samples obtained by $^\bullet$OH-based EF treatment; the morphological analysis was carried out via scanning electron microscope (SEM), the chemical composition was determined by energy-dispersive X-ray (EDX). Details of these methods are described in the following sub-sections.

2.1. Electrochemical-Fenton treatment

In this work, a food industry wastewater producing vinegar was used with a COD level of 10,000 mg/L. The wastewater characteristics are given in Table 1. $^\bullet$OH-based EF process was carried out in a reactor with six mono-polar parallel iron plate electrodes producing different current densities through 400 mL wastewater improved by different H_2O_2 (Merck, Germany) and Na_2SO_4 (Merck, Germany) concentrations.

In order to determine the removal efficiency, the utilization of chemical oxygen demand (COD) was investigated for the determination of $^\bullet$OH-based EF treatment efficiency for the vinegar industry, which had acidic characteristics and carried a high organic pollution load. The utilized experimental set is as shown in Fig. 1.

In Fig. 1, the iron electrode set consists of 6 equal electrodes with 0.3 cm thickness, 4 cm width and 6 cm high. The set vertically immersed area of both anode and cathode electrodes was 100 cm^2 at 4 cm depth with a between-electrode distance of 0.3 cm. Three connected iron electrodes are used as anodes while others are used as cathodes. A current-controlled DC power supply (Statron

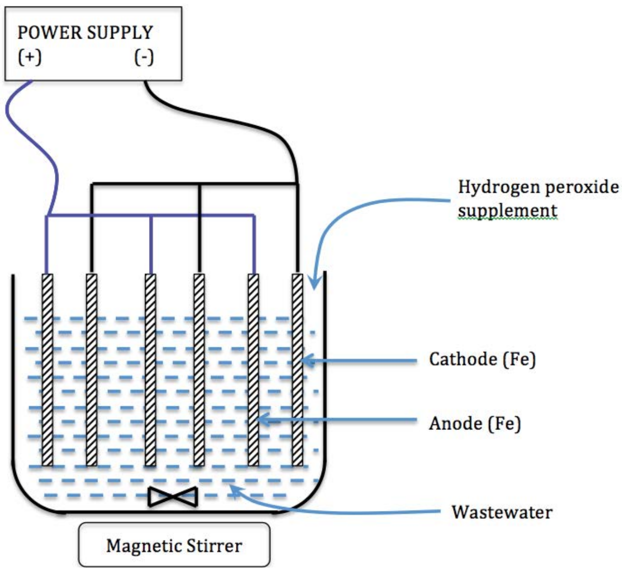

Fig. 1. Experimental setup used in the electrochemical-Fenton (EF) treatment.

Table 1
Wastewater characteristics

Parameter	Value
Chemical oxygen demand, COD (mg/L)	10,000.00 ± 50.00
Initial pH	4.11 ± 0.30
Conductivity (μS/cm)	1,542.00 ± 35.50

type 3262, 0–5 A/0–80 V, Germany) was used to apply constant current (mA) to the electrode surfaces. The pH and conductivity of the wastewater were measured using a pH and conductivity meter (Thermo Scientific Orion STAR A215 model, USA). The experiments were conducted constantly at room temperature with a cylindrical glass reactor of 500 mL on a magnetic stirrer (FALC Instruments F60 model, Italy) working at a stirring speed of 300 rpm. In the EF process, the samples were taken at 10 min. time intervals for measuring their COD values during retention time with 60 min with batch flow condition. The COD values of influent and effluent samples were determined according to TS 2789 ISO 6060 water quality – determination of chemical oxygen demand standard using a thermoreactor (Merck TR 420, Germany) after diluted acidified potassium permanganate solution addition to preventing H_2O_2 interference with COD [18]. At the end of treatment, the percentage of COD reduction was determined as:

$$\text{Removal of COD \%} = \left[\frac{(C_0 - C)}{C_0}\right] \times 100 \tag{22}$$

where C_0 = Initial COD density (mg/L), and C = COD density (mg/L) for retained samples.

In $^{\bullet}$OH-based EF treatment, the determination of energy consumption was determined in parallel with the COD utilization. The consumed energy during the process was calculated alternatively as kWh/kg or kWh/m^3 according to the following equations.

$$\text{Energy consumption}\left(\frac{\text{kWh}}{\text{kg}}\right) = \frac{(V \times I \times t)}{(C_0 - C)} \times \vartheta, \tag{23}$$

$$\text{Energy consumption}\left(\frac{\text{kWh}}{\text{m}^3}\right) = \frac{V \times I \times t}{\vartheta}, \tag{24}$$

where I = current (A), V = voltage (V), ϑ = liquid sample volume (L), t = time (h), C_0 = initial COD density, C = the COD density at time t.

The 400 mL volume of the wastewater with 10,000 mg/L COD value was treated by EF method with the current density (20.00 and 22.5 mA/cm^2), the addition of sodium sulfate as the support electrolyte concentrations (5 mM Na_2SO_4), and H_2O_2 concentrations (30 and 60 mM) according to factorial design randomly.

In $^{\bullet}$OH-based EF treatment, response surface methodology (RSM) was used for the optimization of electrochemical treatment processes parameters in order to modeling and analyzing to these parameters to show linear and non-linear interactions of the treatment parameters. A fractional design of the experiment with 3 factors and 2 levels was made for RSM. The parameters affecting the $^{\bullet}$OH-based EF treatment, their code and their levels used in $^{\bullet}$OH-based EF process were given in Table 2. The parameters were desired as the current density (mA/cm^2), Na_2SO_4 concentration (mM) and H_2O_2 concentration (mM). Minitab 19.0 was used for RSM statistical technique, and the Pareto chart, surface plot/counter plot and regression equation were obtained from RSM analysis.

2.2. Genotoxicity assays

The toxicity evaluation based on observation of effects on *Salmonella typhimurium* bacteria was performed for vinegar industry wastewater. Particularly, the umuC Easy AQ kit was used according to the ISO 13829 standard. The microplate spectrophotometer (Epoch BioTek, Germany) was used for the samples to perform 1 d for the genotoxicity test.

In the umuC AQ (ICT-ANIARA) procedure, *Salmonella typhimurium* TA1535 [pSK1002] bacteria in the exponential growth phase were exposed for 120 min to 4 varying concentrations of each test sample with a positive and negative control. After 2 h, the exposure cultures were diluted in a fresh medium and allowed to grow for another 2 h. The induction and expression of the umuC – lacZ reporter gene were then assessed after lysis of the bacteria. Colorless ONPG was converted to the yellow product *o*-nitrophenol in the presence of induced β-galactosidase (lacZ). The intensity of the color correlated with the amount of β-galactosidase present and thus with the genotoxic potency of the test compound. Measurement of the OD_{600} before and after the 2 h growth phase allowed to calculate of an induction ratio (IR) and to identify toxic growth inhibitory effects. The genotoxic potential of substances was assessed directly and in the presence of liver S9 fractions.

Salmonella typhimurium TA1535 [pSK1002] bacteria in the exponential growth phase were exposed for 120 min to 4 concentrations of a test sample in the Innova 42 Shaker Series incubator. Measurement of the OD_{600} before and after the 2 h growth phase was performed with a microplate spectrometer. An induction ratio (IR) and toxic growth inhibitory effects were calculated via the ICT-ANIARA umuC AQ excel calculation sheet.

2.3. Sludge characterization

The characterization of the resultant sludge samples was via morphological analysis that was carried out by scanning electron microscope (SEM, Zeiss SUPRA). Then, the chemical composition was determined by an energy-dispersive X-ray (EDX, Bruker).

3. Results

In this work, the process conditions of $^{\bullet}$OH-based EF treatment were optimized for the food industry wastewater producing vinegar applying RSM, and the toxicity analysis was performed with umuC genotoxicity assay. The morphological characterization of resultant sludge was performed by scanning electron microscope, and

Table 2
Factorial design of $^{\bullet}$OH-based EF experiment for RSM

Factor	Factor code	Factor level	
		Low	High
Current density (mA/cm^2)	*A*	20.00	22.50
Na_2SO_4 concentration (mM)	*B*	0.00	5.00
H_2O_2 concentration (mM)	*C*	30.00	60.00

finally, the chemical characterization was performed by energy-dispersive X-ray.

3.1. Results of EF treatment

The treatment results of •OH-based EF method are summarized in Table 3 with the zero-order reaction constant. The reaction constant was determined with linear regression coefficient (R^2) according to the following equation:

$$\text{Reaction rate} = \frac{dC}{dt} = k \qquad (25)$$

where dC/dt is the COD removal rate (mg COD/L h) and k is the zero-order reaction constant (mg COD/L h). After the EF treatment, the obtaining COD removal efficiency and the energy consumption vs. time are shown in Fig. 2.

As can be seen in Table 1 and Fig. 2, the wastewater of the food industry producing vinegar with a high COD concentration was successfully treated for the first time in the literature with the EF method. With the EF method, 99% COD removal was achieved with 36.17 kWh/m^3 equal to 39.375 kWh/kg energy consumption in optimum conditions (20 mA/cm^2 current density, 60 mM H_2O_2 and 5 mM Na_2SO_4) after 60 min treatment of wastewater. The COD reduction mechanism of wastewater was explained as zero-order reaction kinetics in the EF treatment. Singh and Mondal investigated the effects of initial pH, current density, hydraulic retention time, electrode distance and supporting electrolyte concentration on the heavy metal removal considering the operating cost of EC. They obtained the 98.51% pollutant removal under the optimum treatment conditions with 10 A/m^2 current density, 1 cm inter-electrode distance, 0.71 g/l NaCl concentration at pH:7 with an operating cost of 0.357 USD/m^3 treated water. [19]. In a similar study, Can et al. [20] reported 99.68% of pollutant removal efficiency at 1.07 mA/cm^2 current density application resulting in 1.23 kWh/m^3 energy consumption at the end of 30 min EC treatment with iron (Fe) electrode. Comparing energy consumption results in this presented study with literature. The high electrical energy consumption with increasing current density was an expected result because higher current density caused to solve more electrode material and remove more pollutants. Also, the energy consumption behavior in the electrochemical treatment mechanism is highly dependent on the chemistry of the aqueous medium, particularly its conductivity and electrochemical reactor design. In addition, parameters such as pH, particle size and concentration of chemical components of wastewater are effective on energy consumption in the electrochemical process.

Lots of researchers reported the reaction kinetics of electrochemical treatment methods for different wastewaters characteristics and varying treatment conditions. Güven et al. [21] studied the electrochemical treatment of beet sugar industry wastewater using iron electrodes, and they investigated that the first-order reaction supplied a good fit. Dirany et al. [22] found that first-order reaction kinetic occurred in EF treatment under the optimum treatment conditions (50 mM Na_2SO_4 and 0.2 mM Fe^{2+}, at 60 mA, pH 3.0 using a Pt/carbon-electrodes). Li and Liu [23] investigated the electrochemical oxidation of ammonia removal with RuO_2/Ti electrodes, and they reported that zero-order kinetics was determined in electrochemical oxidation with Cl^- as a result of indirect oxidation of HOCI. The reaction rate constant was found as 12.3 mg N/L h in the oxidation conditions (3.8–5.4 mA/cm^2 of current density and 30–300 mg/L of Cl^-). Similarly, Lin et al. reported that first-order reaction kinetic was reasonable for phenolic wastewater treatment with electrochemical oxidation depending on the concentration of H_2O_2 [24]. Montanaro and Petrucci [25] worked on the electrooxidation of the Remazol Brilliant Blue reactive dye removal from model wastewater using boron-doped diamond anode, and they observed that the zero-order reaction decay occurred for color removal lower than 150 mg/L dye concentrations. Fukunaga et al. [26] reported that the transition from zero-order kinetics to first-order kinetics related to current density increasing when they treated the wastewater contains formaldehyde using electrooxidation with metal oxides-Ti/$Ru_{0.3}Ti_{0.7}O_2$ electrodes. The reaction kinetics found in our study was compatible with the literature when compared to the electrooxidation studies. When electrochemical treatment studies are examined, it is seen that the degradation mechanism changes depending on the experimental parameters.

In this study, it was determined that the reaction rate of the COD reduction with •OH-based EF method was

Table 3
The treatment results of •OH-based EF method

Run order	Current density (mA/cm^2)	H_2O_2 concentration (mM)	Na_2SO_4 concentration (mM)	Removal efficiency (%)	Energy consumption (kWh/m^3)	Reaction constant (kg/L h)	R^2
1	22.50	30.00	5.00	95.00	38.18	0.0086	0.9979
2	20.00	60.00	5.00	99.00	36.17	0.0089	0.9985
3	22.50	30.00	0.00	92.74	36.94	0.0086	0.9754
4	22.50	60.00	0.00	92.19	38.45	0.0084	0.9769
5	20.00	60.00	0.00	93.00	38.75	0.0086	0.9766
6	20.00	30.00	0.00	92.20	36.17	0.0088	0.9900
7	22.50	60.00	5.00	88.29	39.37	0.0087	0.9968
8	20.00	30.00	5.00	98.05	37.96	0.0088	0.9900

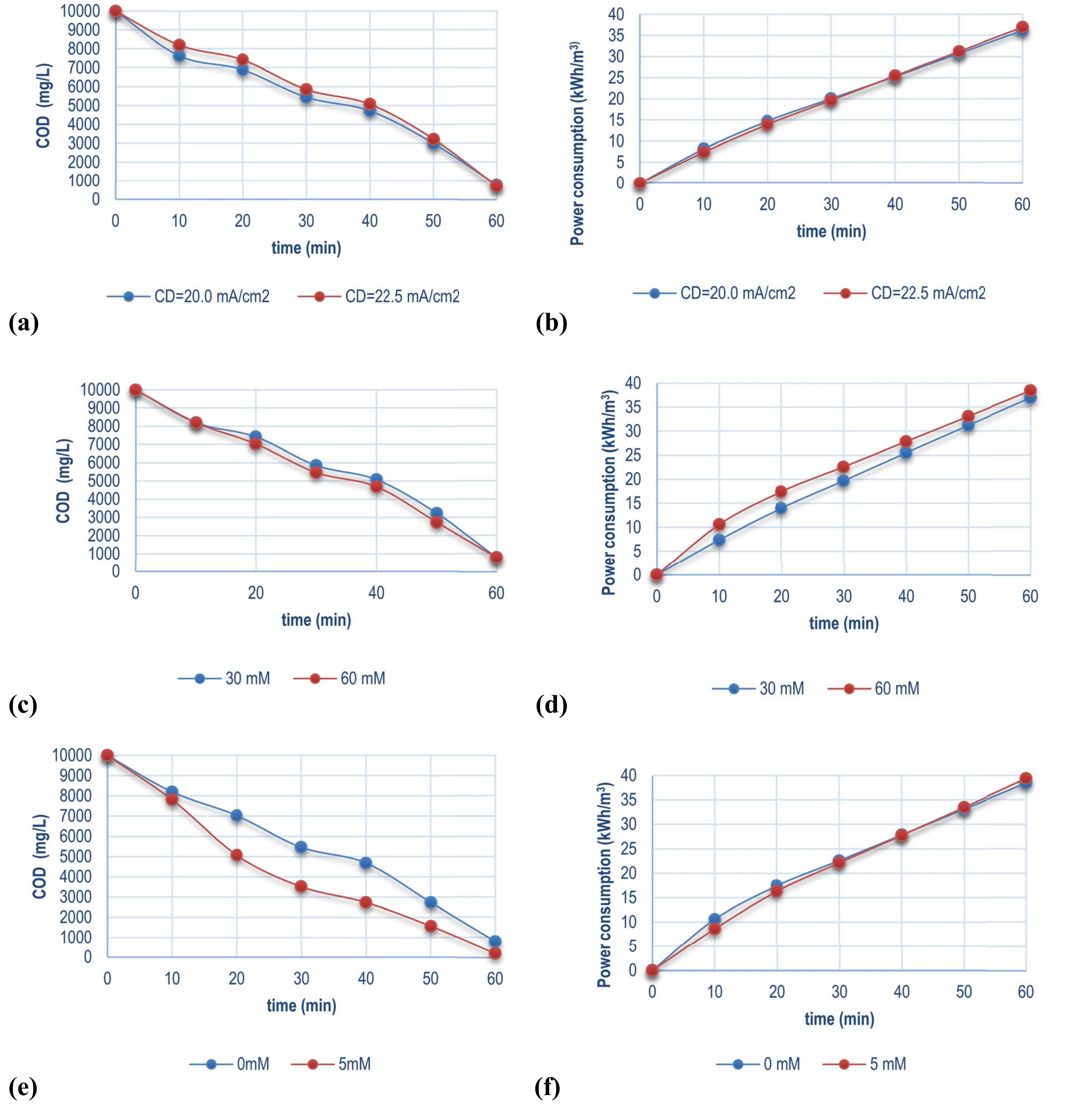

Fig. 2. COD removal efficiency and energy consumption of •OH-based EF method: (a) the current density effect on the COD removal efficiency of i_1 = 20 mA/cm² and i_2 = 22.5 mA/cm²; (b) current density effect on energy consumption of i_1 = 20 mA/cm² and i_2 = 22.5 mA/cm²; (c) the effect of H_2O_2 concentration on COD removal efficiency with 30 and 60 mM; (d) the effect of H_2O_2 concentration on energy consumption with 30 and 60 mM; (e) the effect of Na_2SO_4 concentration on COD removal efficiency with 0 and 5 mM, (f and e) the effect of Na_2SO_4 concentration on energy consumption with 0 and 5 mM (C_0 = 10,000 mg COD/L, $pH_{initial}$ = 3.96, conductivity ≈ 1,542 µS/cm, the pilot referred the average result of three independent trials performed for each parameter).

associated with current density, the concentration of H_2O_2 and the concentration of Na_2SO_4 in Table 2. It was clearly stated that the double and triple effects of these electrochemical treatment parameters, and which parameters were more effective on COD removal of this wastewater in the Pareto chart given in Fig. 3. The regression equation expressing the effects of these parameters on removal efficiency is given in Eq. (12) where A is the current density (mA/cm²), B is the concentration of H_2O_2 (mg/L), and C is the supporting electrolyte concentration of Na_2SO_4 (mg/L).

$$\text{COD Removal Efficiency (\%)} = 76.30 + 0.7552A + 0.3861B - 3.226C - 0.01797A \times B + 0.2182A \times C + 0.3379B \times C - 0.01684A \times B \times C \tag{26}$$

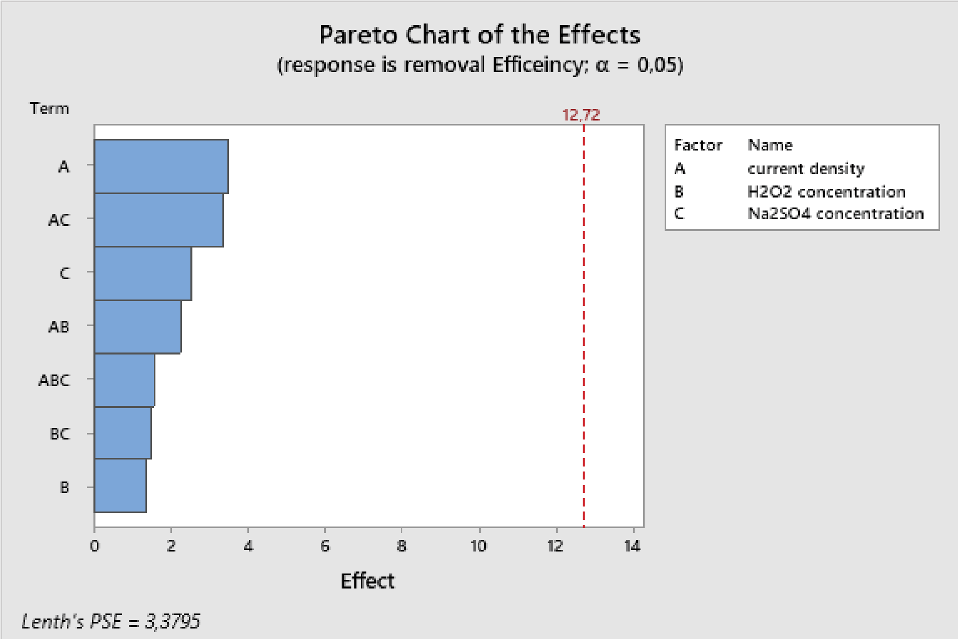

Fig. 3. Pareto chart of EF process parameter on COD removal efficiency.

Therefore, the regression equation with coded values of the •OH-based EF treatment parameters was given in terms of current density (*A*), the concentration of H_2O_2 (*B*), and Na_2SO_4 (*C*) to express in a more understandable way. It was determined that EF treatment of the wastewater included the linear terms of the current density (*A*), and the concentration of H_2O_2 (*B*) and Na_2SO_4 (*C*) effecting the COD removal response with the double ($A \times B$, $A \times C$, and $B \times C$) and triple effects ($A \times B \times C$) of these EF treatment parameters.

The initial level of current density was selected as 20.00 mA/cm^2 to attain enough Fe^{2+} amount dissolved from the anode passing a constant current. In electrochemical treatment studies, it is desired to keep energy consumption to a minimum by applying minimum current density obtaining the required pollutant removal efficiency. It is also known that the amount of sludge formed increases with increasing current density. For this purpose, the current density values were increased with as small increments as possible. Also, the effect of electrochemical treatment parameters such as current density on the removal efficiency should be optimized with a method such as RSM. In consequence, the RSM results showed that the most effective parameter was the current density, even at the current density values increased with these small increments according to Fig. 3. It could be indicated that the current density determined the coagulant dosage rate, the bubble production rate, the size and the growth of flocs.

The surface plot and the contour plot of the COD removal as a function of the current density vs. H_2O_2 concentration (a), and current density vs. Na_2SO_4 concentration (b) are shown in Fig. 4 obtained from RSM analysis with the fractional design of EF treatment.

As a result of RSM analysis with the fractional design of •OH-based EF treatment, it was investigated that the most effective parameter of EF treatment was the current density. The applied current density should be chosen at the minimum value that will allow sufficient Fe^{2+} dissolution. It was observed that sufficient dissolution on the electrode surface did not occur, since the required threshold energy for electrochemical treatment could not be provided below 20 mA/cm^2 current density for the used electrode assembly and this type of wastewater characteristics according to the preliminary tests. Therefore, the lower current densities were not studied in the presented manuscript. The main reason why the current density was the most effective parameter of them was thought that the current density played an effective role in the both the amount of H_2O_2 formed in-situ and the Fe^{2+} dissolved from the anode passing current [27]. The current density played a more effective role on COD removal than other treatment parameters since both reactants were produced in the system with increasing current density simultaneously. Consequently, the increasing current density affected the Fe^{2+} ions generation rate and raising the concentration of H_2O_2.

The current density played an effective role in both the amount of H_2O_2 formed in-situ and the Fe^{2+} dissolved from the anode passing current. Therefore, EF reaction occurs even in Fe-electrocoagulation. It is commonly preferred to add H_2O_2 externally in EF studies because the high current density and long contact time can be required for in-situ production of H_2O_2 expressed as •OH-based EF in the literature. In the presented study, the minimum H_2O_2 concentration that should be added to the EF system to examine the effect of the H_2O_2 concentration was determined as 30 and 60 mM as a result of the preliminary studies. RSM results also showed that the least effective parameter in the EF system was the H_2O_2 concentration. It could also be concluded that the in-situ production of H_2O_2 is sufficient for this type of wastewater.

3.2. Results of genotoxicity assays

The β-galactose induction ratios, which indicate the occurrence of DNA damage of *Salmonella typhimurium* TA1535/pSK1002 bacteria exposed to treated and untreated wastewaters were identified in the genotoxicity assays. The results of genotoxicity assays were shown in Table 4 when the dilution factor was equal to 1.5, which means that the sample was diluted 1.5 times. The effect of the dilution factor on the β-galactose induction ratios of untreated wastewater is shown in Fig. 5 comparing the induction ratio threshold.

The results in Table 3 and Fig. 2 constitute proof of the efficiency and usability of •OH-based EF method for the treatment of food industry wastewater producing vinegar. This type of wastewater naturally contains complex organics, forming an acidic medium. The proposed •OH-based EF method yields a high COD removal ratio within 60 min of treatment. Although this level of treatment is very satisfactory, a usability analysis of the treated wastewater by means of genotoxicity tests is necessary. Such analysis is crucial due to the possibility of treated wastewater containing genotoxins that might have adverse effects on human health and the ecosystem. In that aspect, biological treatment might be a clean alternative for its minimal environmental impact. However, it is known to be slow and ineffective in wastewater with a high level of organic matter with acidic character. This type of wastewater is such an example with a high level of COD and phenolic compound, making it an acidic medium. Acidic media are naturally difficult for biological processes and they reduce its effectiveness [28]. Therefore, biological treatment is not a reasonable alternative for vinegar wastewater treatment. As a result, •OH-based EF method stands to be a plausible tool with its high efficiency. The genotoxicity test results with β-galactose induction ratio value below 1.5 confirm that compliance of •OH-based EF method with the limits necessary for ICT-ANIARA umuC AQ procedure. Consequently, •OH-based EF method is observed to be both effective in COD removal and toxicity removal in yielding usable and eco-friendly treated wastewater.

Several bacteria-based test systems are developed for toxicity level measurement. Due to being fast, precise

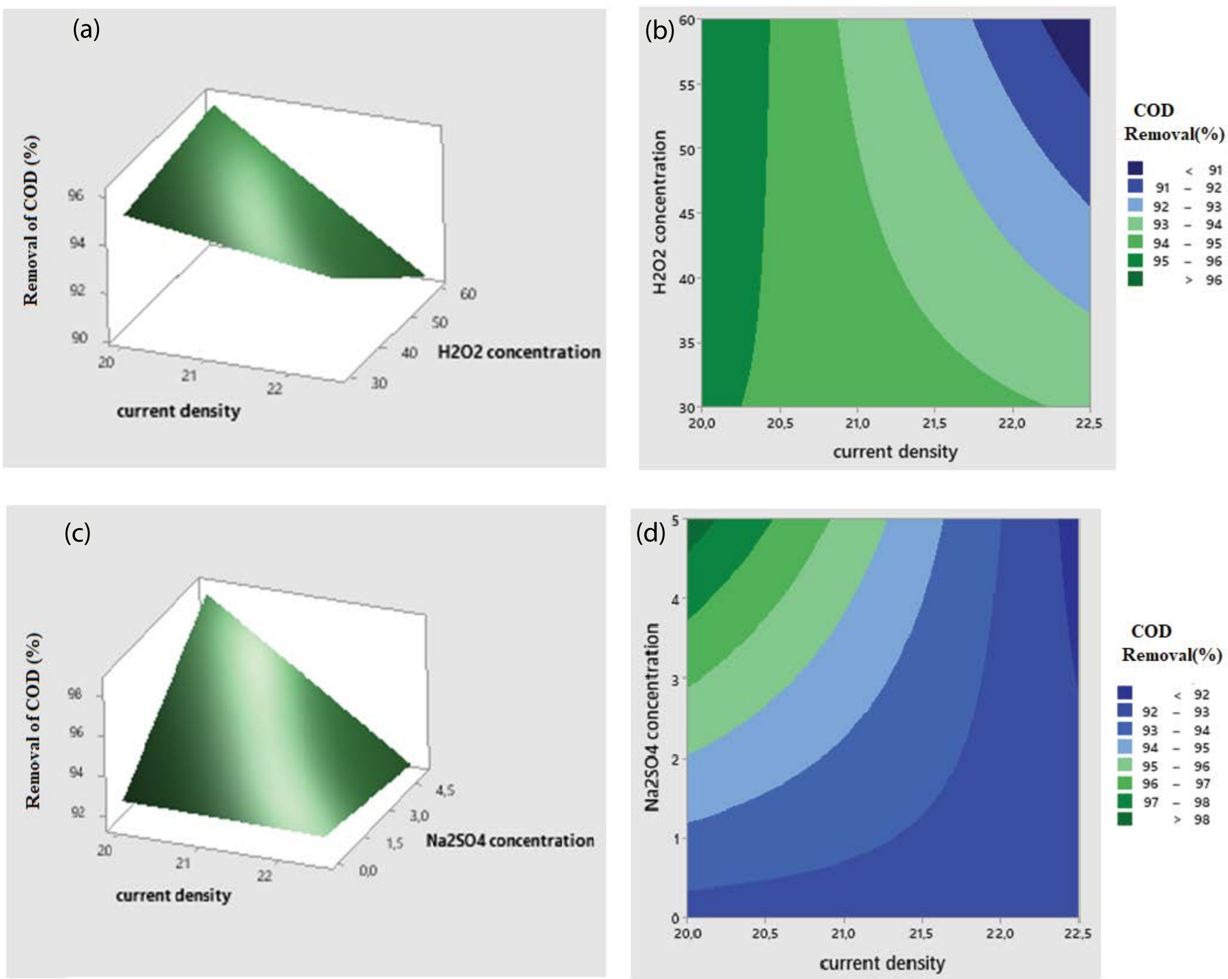

Fig. 4. The surface plot (a) and the contour plot (b) of the COD removal response as a function of the current density vs. H_2O_2 concentration, and the surface plot (c) and the contour plot (d) of the COD removal response as a function of and current density vs. Na_2SO_4 concentration.

Table 4
The results of genotoxicity assays

Sample	Dilution factor	Growth factor *G* (Units)	Relative β-galactosidase activity US (Units)	Induction ratio IR (Units)	Induction ratio of test limit IR (Units)	Genotoxicity characterization
Untreated wastewater (–S9)	1.5	0.838	7.570	10.494	1.500	Toxic[a]
Untreated wastewater (+S9)	1.5	0.791	6.708	10.795	1.500	Toxic[a]
Treated wastewater (–S9)	1.5	0.953	0.997	1.382	1.500	Non-toxic[a]
Treated wastewater (+S9)	1.5	0.736	1.114	1.793	1.500	Toxic[a]

[a]ICT-ANIARA umuC AQ procedure test limit for genotoxicity characterization

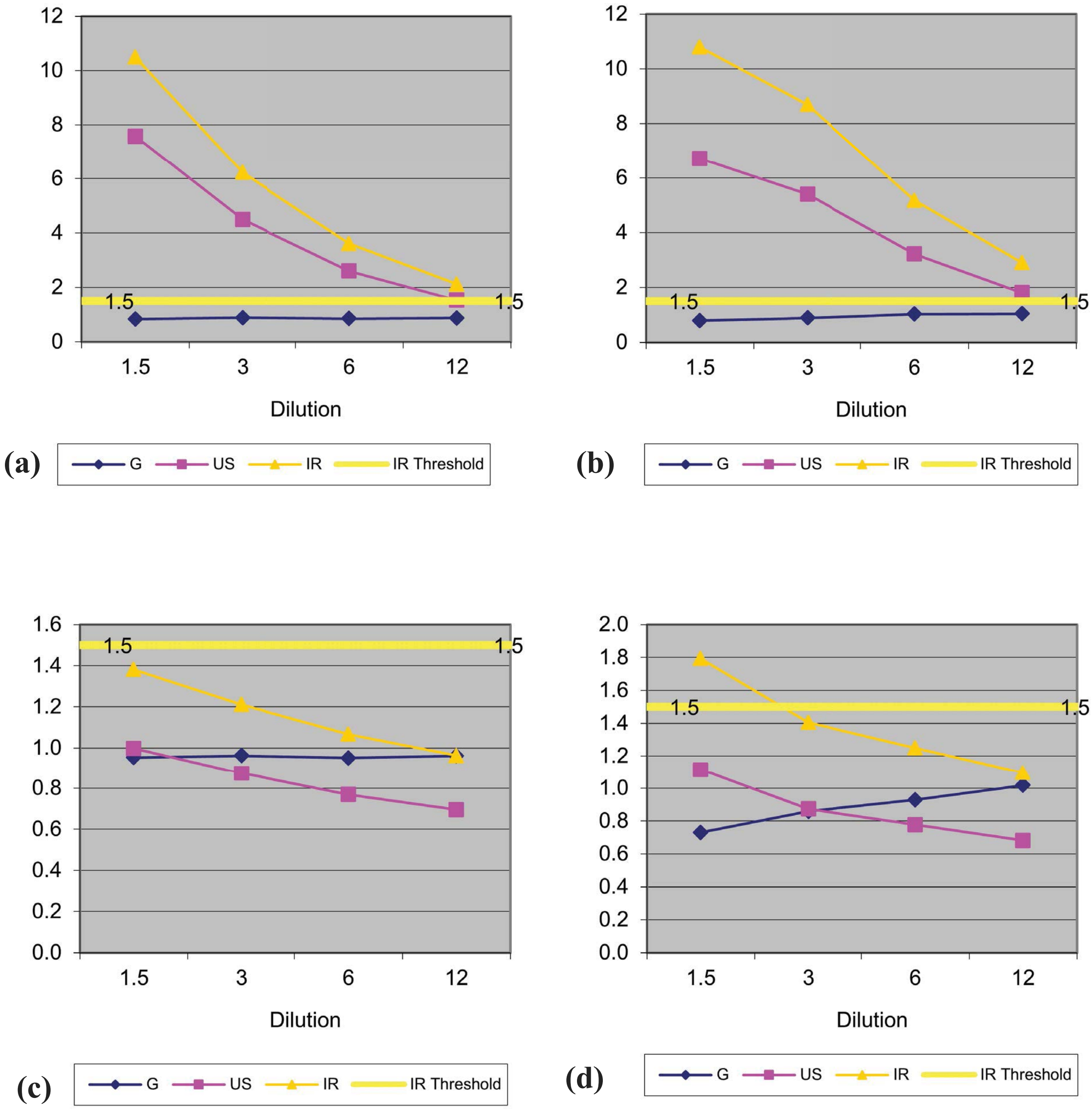

Fig. 5. The effect of dilution factor on the β-galactose induction ratios of the untreated wastewater without S9 (a), and with S9 (b), the β-galactose induction ratios of treated wastewater without S9 co-factor (c) and with S9 co-factor (d).

and economical, several bacterial bio-experiments are standardized. Hernando et al. [29] have monitored the applicability and validations of such test methods for wastewaters discharged to wetlands; by means of bacterial bioluminescence, Daphnia test and algae growth inhibition tests. Clearly, toxicity may also be due to solvents in the process (yielding metals such as Cr, Cu, Cd, Ni and Zn) [30], as well as due to advanced oxidation. Related literature reveals that even standardized modern treatment methods for industrial wastewater with toxic content are not capable of eliminating the toxicity literature survey reveals that even modern treatment standards are incapable of eliminating toxic effects of the output over the ecosystem. Consequently, any proposal of a novel treatment method should carefully consider the toxicity at its output. Besides, the test for toxicity should also be chosen carefully to satisfy the efficiency and cost conditions. In a work by Gert-Jan de Maagd and Tonkes [31], it was observed that bacteria assays resulting from SOS value indicating emergency response in the displayed toxicity assay give an efficient genotoxicity medium for wastewater analysis, therefore this genotoxicity method was adopted from the summarized literature above. Also, the fast response bacterial-based toxicity assays were used to determine the genotoxic effects of persistent organic compounds especially non-biodegradable micropollutants in the surface water and in the different types of wastewater [32]. The umuC and Ames assays were the most preferred foreword genotoxicity tests in the literature for this purpose, and these assays were used for the genotoxic effect of drugs, antibiotics, detergents, disinfectants, and their by-products in especially hospital wastewater [33]. When it comes to determining the genotoxic impact of food industry wastewater varying characters of mixed and complex pollutants were not obviously identified in previous studies. Hartmann et al. [33] studied the identification of the genotoxic effect of the selected antibiotics in hospital wastewater, and they found a correlation between umuC IR factor and the concentration of selected antibiotics. Giuliani et al. [34] investigated the genotoxic character of hospital wastewater with umuC test, and they reported that nearly 15% of the collected samples during 2 y showed DNA damaging effect on test bacteria. These studies were stated that the umuC assays were a short-term, facile and screening tool for wastewater genotoxicity assessment. Žegura et al. [35] determined the genotoxic and cytotoxic potential of wastewater, surface water and drinking water bodies using umuC assays with *Salmonella typhimurium* TA1535/pSK1002 bacteria and mammalian cell test (MMT) with human hepatoma cells. They stated that these water and wastewater samples had cytotoxic and genotoxic effect and their toxic character were caused by the pharmaceutical and inflammatory drug and disinfectants. They reported that the umuC assays were an identical tool for genotoxicity screening of water samples giving a useful combination of these toxicity tests to detect the ecotoxicological effect of micro-pollutants found in water and wastewater. In addition, it was stated that the genotoxic effect was a more distinctive and reasonable indicator of ecotoxicity in many studies, in which the cytotoxic and genotoxic potential was examined simultaneously for surface water and wastewater effluents. Consequently, our findings gave the first holistic approach in the literature because it was the unique study where COD removal and toxicity removal were examined simultaneously with EF advanced treatment method.

3.3. Results of sludge characterization

For the characterization of sludge that is produced by the $^{\bullet}OH$-based EF treatment process; scanning electron microscope (SEM) images are presented for morphological analysis, and EDX results are presented for the chemical compositions. The sludge characterizations were also performed before and after $^{\bullet}OH$-based EF treatment of the wastewater. EDX results of the untreated and treated wastewater sludge are given in Table 5, and their corresponding spectrum is shown in Fig. 6.

Table 5
EDX results of untreated vinegar wastewater samples

Sample	Element	Mass ratio (%)	Atomic (%)	Compound (%)	Compound formula
Untreated wastewater sludge	C	24.63	31.24	90.26	CO_2
	Na	1.02	0.67	1.37	Na_2O
	Mg	0.49	0.30	0.80	MgO
	P	0.52	0.26	1.19	P_2O_5
	S	0.97	0.46	2.43	SO_3
	Cl	0.66	0.28	0.00	–
	K	2.44	0.91	2.82	K_2O
	Ca	0.33	0.13	0.47	CaO
	O	69.04	65.74	–	–
$^{\bullet}OH$-based EF treated wastewater sludge	S	3.93	4.07	9.82	SO_3
	K	1.90	1.62	2.29	K_2O
	Fe	68.32	40.64	87.89	FeO
	O	25.85	53.67	–	–

According to the EDX result given in Table 3 and EDX spectrum for untreated wastewater sludge and •OH-based EF treated wastewater sludge in Fig. 6, the results of the EDX analysis were performed to examine the elemental constituents of sludge provided direct evidence that organic compounds found in wastewater removed from waste sludge. After the •OH-based EF treatment, other elements detected in sludge come from wastewater ingredients and supporting electrolyte solution. Similar sludge characterization studies were carried out since the properties of the sludge formed after treatment would determine the disposal or sludge treatment methods and their cost. Vasudevan et al. [36] examined the chromium content on sludge formed after electrochemical coagulation for model chromium wastewater to determine the toxic sludge generation due to the nature of heavy metals. Although less sludge formation is listed among the advantages of the •OH-based EF method, elemental characterization of formed sludge would be necessary as it could be a possible option to reuse that sludge in the appropriate area instead of its treatment or disposal. In this aspect, our study contained important results. Gönder et al. [37] reported that hydroxides and oxides compound negatively charged with a wide range of pH in the sludge samples were occurred after electrocoagulation using Fe–Al electrode of carwash wastewater. Drouiche et al. [38] investigated the sludge characteristic that occurred after electrocoagulation using bipolar iron electrodes for removal of fluoride from polishing wastewater, they reported the composition of sludge was formed almost 50% iron due to particle destabilization and oxidation. In our study, Fe^{2+} ion dissolved from electrode surface converted iron oxide in sludge according to EDX results. The SEM images of sludge for the untreated and •OH-based EF treated wastewater are shown in Fig. 7.

Corresponding SEM microscopic images are given in Fig. 7. The iron oxide particles clearly show an agglomerated formation of these oxide particles in the settled sludge after •OH-based EF treatment of food industry wastewater. The SEM images clearly reflect the relative sizes of the iron oxide particles with a 68.32% mass ratio in sludge. Drouiche et al. [38] showed that iron oxide particles were observed with two different shapes aggregated as sphere and prism geometrical types in SEM images. Similarly, Lai and Lin [24] illustrated the SEM images of particles agglomerated as oxide from giving their sizes in sludge comparing the raw wastewater with wastewater after electrocoagulation. Subsequently, the iron oxide particles smaller than 2 μm size seen in Fig. 7 made it possible to consider the reuse of this sludge formed as a result of •OH-based EF treatment in the proper area.

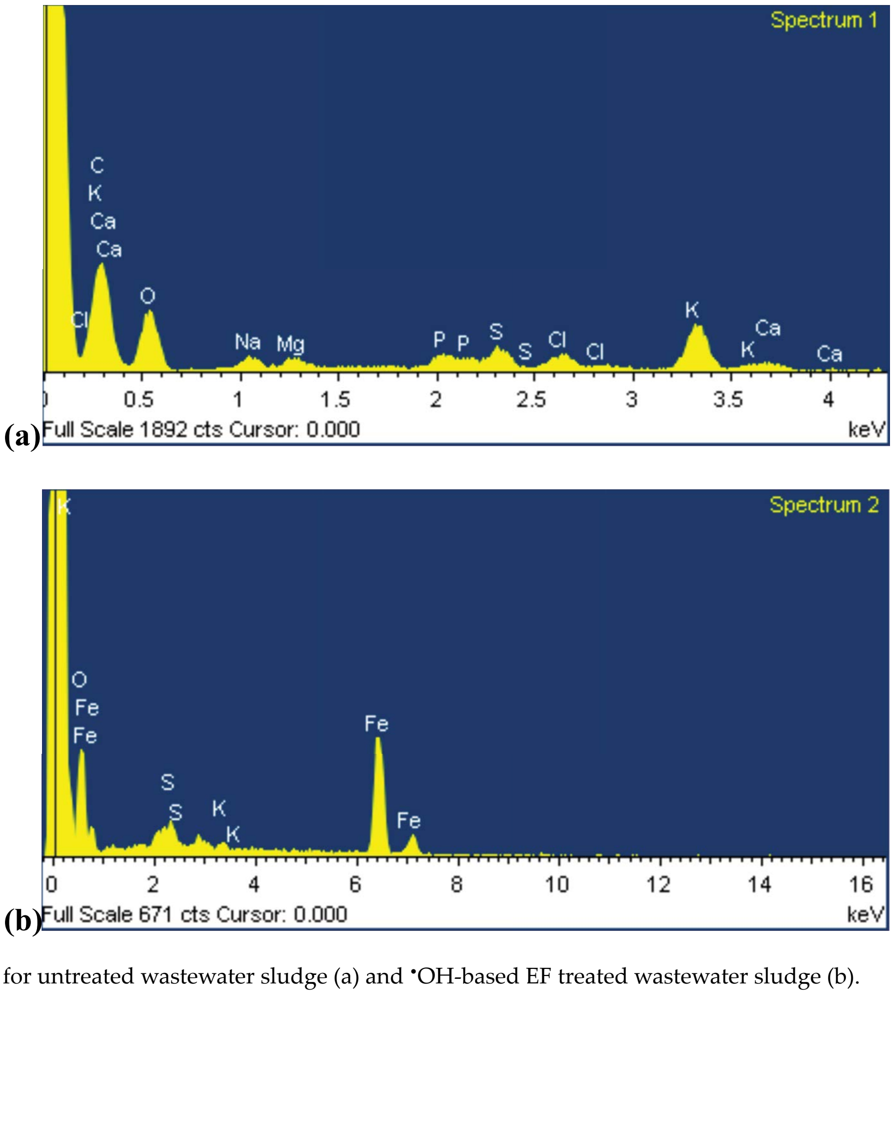

Fig. 6. EDX spectrum for untreated wastewater sludge (a) and •OH-based EF treated wastewater sludge (b).

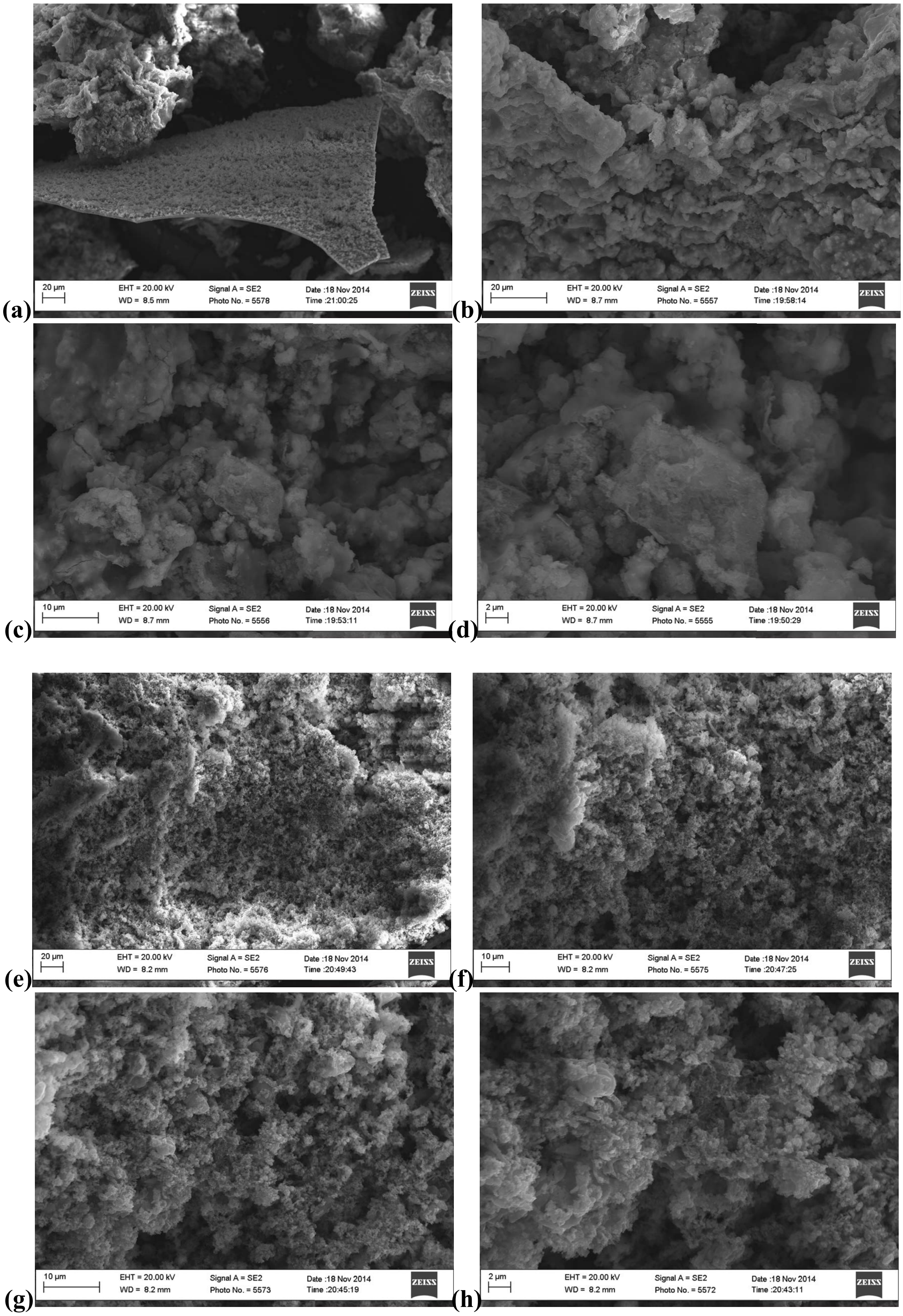

Fig. 7. SEM images of sludge samples (a) 1,000x; (b) 2,500x; (c) 5,000x; (d) 10,000x magnification of untreated wastewater sludge, (e) 1,000x; (f) 2,500x; (g) 5,000x; (h) 10,000x magnification of •OH-based EF treated wastewater sludge.

4. Conclusion

This work presented the treatment results of food industry wastewater producing vinegar using the •OH-based EF process. This type of wastewater was highly polluted with a high level of acidity, and it was shown that the proposed treatment method was plausibly effective in pollution removal. Besides, genotoxicity tests and evaluations are also carried out over the treated wastewater, and it was observed that the results were well below the toxic level standards. Specifically, the results were summarized in the following list.

- The wastewater with a COD value of about 10,000 mg/L was treated by EF method at an electrical current density of 20.00 mA/cm^2 with the addition of sodium sulfate as the supporting electrolyte at a concentration of 5 mM. Such treatment consumed 36.17 kWh/m^3 energy and treatment efficiency of 99.0% was achieved.
- The zero-order reaction kinetic observed in •OH-based EF removal mechanism with 0.0089 kg/L h rate constant (0.9985 linearities).
- As a result of RSM analysis with the fractional design of •OH-based EF treatment, the most effective parameter of •OH-based EF treatment was the current density because the current density played an effective role in both the amount of H_2O_2 formed in-situ and the Fe^{2+} dissolved from the anode passing current.
- The β-galactose induction ratio of treated wastewater was below 1.5 that is the limit value specified for genotoxicity due to the ICT-ANIARA umuC AQ procedure.
- The results of the EDX analysis performed to examine the elemental constituents of sludge provided direct evidence that organic compounds found in wastewater removed from waste sludge, and Fe^{2+} ion dissolved from electrode surface converted iron oxide in sludge.
- The sludge consisted of agglomerated iron oxide particles smaller than 2 μm in size with a 68.32% mass ratio.

To conclude, this paper illustrated the holistic approach for COD removal and toxicity with •OH-based EF advanced treatment method for food industry wastewater producing vinegar including RSM methods and sludge characterization.

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