

Integrated Treatment of Saline Oily Wastewater Using Sono-Electrokinetic Process, Degradation Mechanism, and Toxicity Assessment



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Integration of sonication (US) with electrokinetic (EK) oxidation was studied for the treatment of a saline oily wastewater, as well as the effect of operating parameters, including pH, voltage, electrode distance (ED), sonication power, and reaction time on COD removal. A COD removal of 98 % was observed for the sono-electrokinetic (SEK) process with an applied voltage of 2.5 V, US power of 300 W, initial COD concentration of 3850 mg L⁻¹, and reaction time of 9 h. The efficiency of SEK over sonication alone and EK oxidation alone was also confirmed with a higher pseudo-first-order reaction rate constant of 0.43 h⁻¹, compared to values of 0.13 and 0.01 for alternative processes. In addition, the biodegradability of effluent was improved based on average oxidation state (AOS) and carbon oxidation state (COS) analysis. Oxygen consumption rate inhibition, dehydrogenase activity inhibition, and growth rate inhibition methods demonstrated the low toxicity of effluent (12–15 %) compared to influent. The current work indicated that SEK is a reliable and efficient technology for the treatment of saline oily wastewaters containing recalcitrant aromatic organics.

Keywords:

saline oily wastewater, electrokinetic oxidation, sonication, toxicity assessment, advanced oxidation technology

Introduction

Petroleum and fuel transportation in ports and the distribution of these hydrocarbon liquids via central terminals into cities, as well as storage of petroleum and its derivative in large tanks, produces a huge amount of oily and saline wastewater.¹ The presence of aromatic and aliphatic hydrocarbons, heavy metals, and high concentrations of minerals represent serious environmental and health risks, which necessitate the application of suitable technologies for the treatment of these flows before discharge into water bodies.²

Research has proved the carcinogenic and mutagenic effects of oily wastewater. Severe adverse effects on water quality, aquatic plants, birds, and

fishes have been reported due to the discharge of untreated oily wastewaters.³ The presence of different organic and inorganic inhibitors, along with high saline content, limits the available alternative for efficient treatment of these wastewaters. Biodegradation is limited due to the destruction and death of biomass in the saline environment and low efficiency of bacterial strain in the degradation of recalcitrant organic pollutants, especially for high hydrocarbon concentrations.^{4,5} Adsorption, membrane filtration, and conventional chemical oxidation are not technically viable options, and they are rejected due to high operating costs. Advanced oxidation processes (AOPs) lead to the creation of highly reactive radicals that are efficient in oxidation and mineralization of recalcitrant organics, even in saline solutions.⁶ AOPs rely on the production of HO• radicals as strong oxidizing agents ($E_0 = 2.8$ V) that oxidize organic molecules efficiently.⁷

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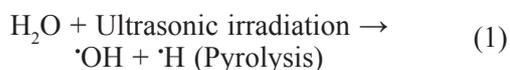
Table 1 – Characteristics of raw saline oily wastewater

Parameter	Mean	STDEV	Max	Min
COD (mg L ⁻¹)	4055	144.3	4300	3850
BOD ₅ (mg L ⁻¹)	965	84.7	1680	376
BOD ₅ /COD	0.23		–	–
TOC (mg L ⁻¹)	3560	257.2	3640	2920
TDS (mg L ⁻¹)	14100	360.8	16400	12300
TSS (mg L ⁻¹)	642	74.6	820	316
pH	7.9	0.89	8.5	7.2

The efficiency and simplicity of AOPs make them a suitable option for the removal of toxic chemicals from wastewaters.^{8–10} Electricity-based reactions, including electrokinetic oxidation (EK), have recently been applied for the oxidation of recalcitrant organics in petrochemical, textile, and tannery wastewaters. EK oxidation has demonstrated benefits, including efficient degradation of organic compounds, cost-effectiveness, low chemical consumption, and low required area compared to other technologies for the treatment of saline and hydrocarbon-containing wastewater. EK degradation is classified into direct and indirect oxidation routes. In addition, for saline wastewater, a direct relation between the reaction rate and conductivity is expected.¹²

The indirect EK oxidation relies on adding mediators such as chlorine and iron salts to oxidize pollutants directly, while direct EK oxidation is dependent on anodes to form strong oxidants, i.e., hydroxyl radicals ($\cdot\text{OH}$) and/or hypochlorite ions. Electrode materials are important in the EK process in which graphite electrodes have been investigated, because of their unique physicochemical characteristics, such as cost-effectiveness, chemical stability, high mechanical strength and conductivity, large specific surface area, and high current efficiency.

EK oxidation can be integrated with ultrasonic irradiation to enhance the oxidation efficiency. Ultrasound is a wave with a frequency higher than 20 kHz.¹¹ Sonication efficiency is affected by chemical structure, volatility, and solubility of the pollutants. The main sonication mechanisms include i) direct pyrolysis of volatile substances in bubbles, and ii) oxidation of non-volatile substances by oxidizing agents (H_2O_2 and $\text{HO}\cdot$) produced through cavitation phenomenon in solution. The overall scheme of reaction is as expressed in Eqs. (1–3):¹³



This integration enhances the degradation of recalcitrant organics in a saline medium of oily wastewaters. According to literature, this is the first investigation on treatment of a real saline hydrocarbon-based wastewater by integrating sonolysis with EK oxidation (SEK). Furthermore, the effect of operating parameters and toxicity of final effluent was studied.

Materials and methods

Materials

Sulfuric acid (H_2SO_4 , 98 %), silver sulfate (Ag_2SO_4 , 99.99 %), mercuric sulfate (99 % HgSO_4 , 99.99 %), potassium dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$, 99.5 %), and sodium hydroxide (NaOH , 99.8 %) were of analytical grade and purchased from Merck Co., Germany. Graphite electrodes as the anode and the cathode with dimensions of $0.5 \times 3 \times 15 \text{ cm}^3$, were purchased from Seraj Corporation, Iran. A saline hydrocarbon-polluted wastewater was prepared from a central fuel distribution terminal in southwestern Iran, and transferred to experimental laboratory using a cold box at $4 \text{ }^\circ\text{C}$ on a weekly basis. Its characteristics, including COD, BOD₅, TDS, TSS, and pH were analyzed. The characteristics of raw saline wastewater are presented in Table 1.

Experimental setup

A cubic glass lab-scale setup ($10 \times 15 \times 5 \text{ cm}^3$) with effective volume of 0.45 L was used to evaluate the treatment of saline oily wastewater at room temperature ($25 \pm 1 \text{ }^\circ\text{C}$). Electrodes were placed in a designed distance from each other inside the reactor, and connected to a laboratory DC power supply with wire (Model: PS 303D, 30 V, 5 A). The voltage could be set using the tuning screw (Fig. 1). The solution was sonicated using a 50 kHz ultrasound generator (Hielscher: UP 400S, Germany), containing a 7 mm titanium probe. The probe tip was placed 20 mm below the surface of the solution and sonicated in an on/off (5 s / 5 s) pulse state in a designed power. Monitoring of the solution pH was performed using HCl 0.5 N or NaOH 0.5 N via a digital pH-meter (Model: Metrohm-827). All experiments were performed in batch mode.

SEK treatment procedure

A portion of a 300-mL real wastewater sample was poured into the reactor. Reactions were initiated after turning on the DC power supply and sonication apparatus. Effects of operating variables, including the initial pH (3–8), voltage (0.5–3 V), electrode distance (ED) (2–10 cm), reaction time (1–9 h), and of the sonication (100–400 W) on COD

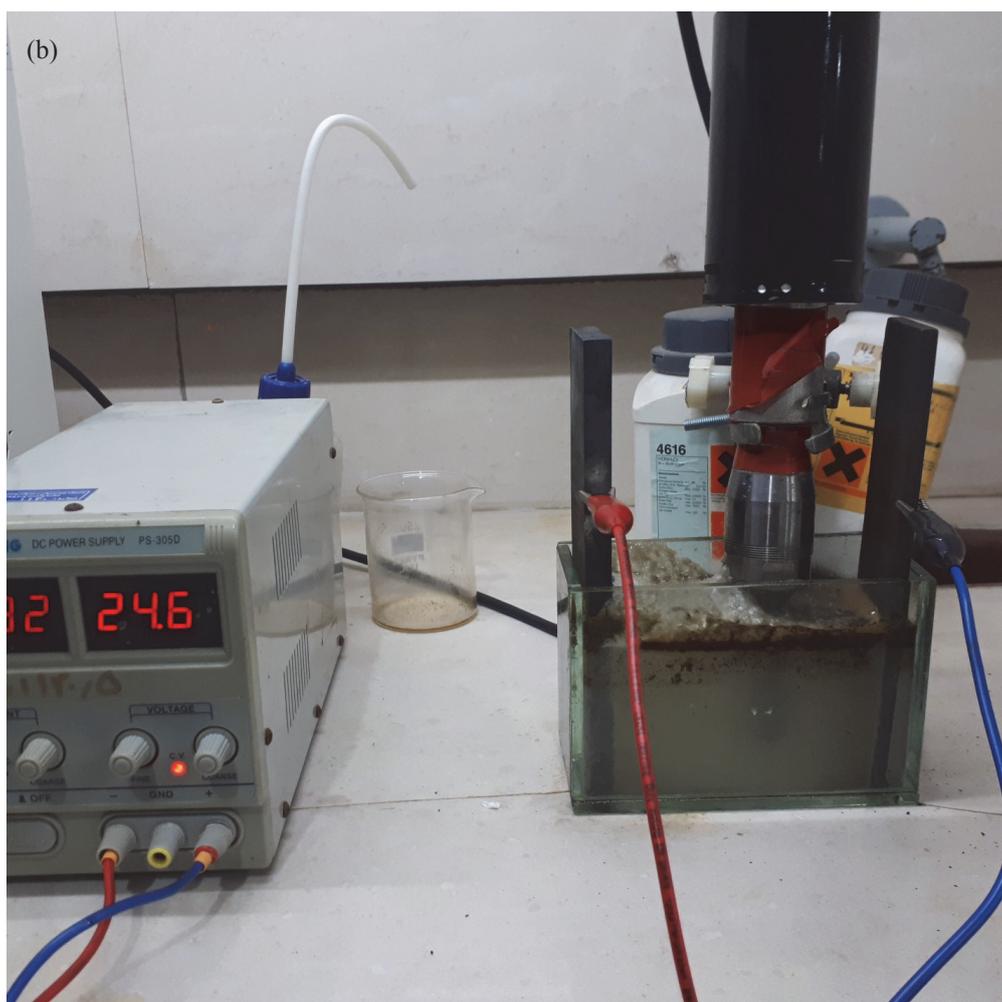
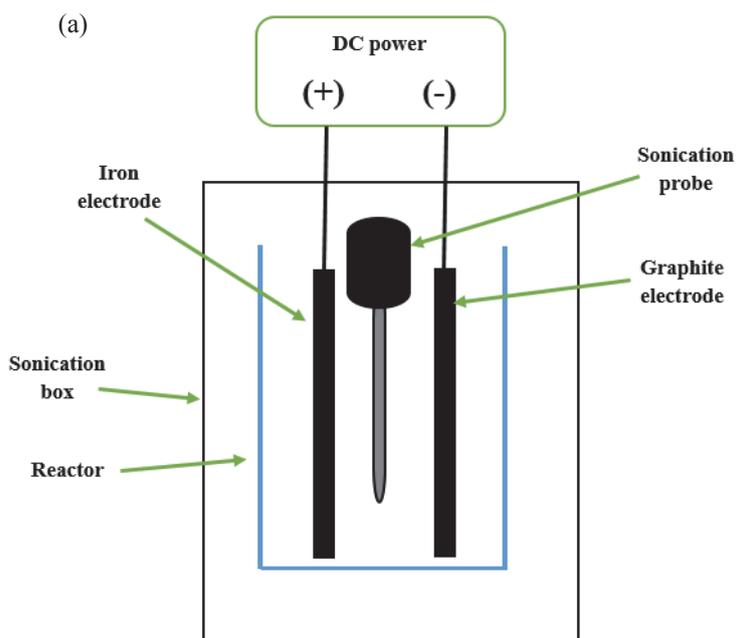


Fig. 1 – a) The schematic of SEK reactor for treatment of saline oily wastewater and b) the pictorial view

removal were investigated based on one factor at the time experimental design. All the experiments were carried out at room temperature (21–25 °C). In addition, the kinetic model and degradation intermediates were analyzed. The process efficiency was calculated based on the COD analysis, while the removal was calculated according to Eq. (4):

$$\text{Removal (\%)} = (C_0 - C_t)/C_0 \cdot 100 \quad (4)$$

where C_0 and C_t represent the concentrations of COD (mg L^{-1}) at the beginning and end of reaction, respectively.

Toxicity assessment

Toxicity evaluation was performed using the oxygen consumption rate inhibition method as described in ISO 8192 (2007),¹⁴ the nitrification rate inhibition method as described in ISO 9509 (2006),¹⁵ and the growth rate inhibition method according to ISO 15522 (1999).¹⁶ Details can be found in supplementary file 1.

Experimental analysis

The specifications of saline oily wastewater for chemical oxygen demand (COD), biochemical oxygen demand (BOD_5), total dissolved solids (TDS), total suspended solids (TSS), and pH were determined based on standard methods for the examination of water and wastewater.¹⁷ A TOC analyzer (Shimadzu, TOC-VCSH, Japan) was applied for the measurement of total organic carbon (TOC). The average current was determined by a SK-7603 Clamp ampere meter. Qualitative analysis of wastewater composition was carried out using GC-MS (model: Agilent 7890, USA) using capillary column HP-5MS (30 mm \times 0.25 mm \times 0.25 mm film thickness, 5 % phenyl 95 % dimethylpolysiloxane stationary phase). The carrier gas (helium) was fed using a steady-state 1 mL min^{-1} flow rate. The temperature of the stove was first adjusted to 40 °C for 1 minute, and then increased to 300 °C at 5 °C min^{-1} . This temperature was maintained for 3 minutes. Finally, the sample was injected into a device at a 10:1 ratio. The final data were reported in terms of the calculated average of at least three replicates.

Statistics

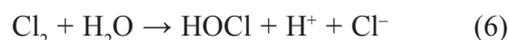
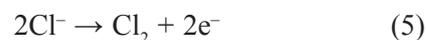
Using SPSS statistics 22 software, the descriptive statistics including mean, standard deviation, maximum values, etc., were analyzed. In addition, the normality of obtained data were examined by Shapiro-Wilk test. Furthermore, a significant difference between results of different levels for each operational variable was investigated using independent sample T-test to interpret the results and selection of the desired level.

Results and discussion

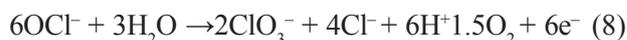
EK oxidation

According to literature, the initial pH of the solution is a key controlling parameter for the oxidation of recalcitrant organics using EK reaction.^{18,19} Results indicated that COD removal through the EK process was highly pH-dependent, and according to Fig. 2a, the COD removal decreased with the increase in initial pH. Results indicated that the highest COD removal efficiencies of 20.4 %, 18.2 %, and 17.3 % were observed for initial pH values of 3, 4, and 5, respectively. In indirect EK process, strong oxidants, such as hypochlorite/chlorine (HClO/ClO), ozone (O_3), and hydrogen peroxide (H_2O_2) are electrochemically generated. The pollutants are then destroyed in the bulk solution by oxidation reaction of the generated oxidant.

The removal efficiency decreased considerably as pH increased to 8. This may be attributed to the fact that, in alkaline conditions, electrolyte would be consumed excessively, resulting reduced conductivity of reaction solution.²⁰ Also, in acidic conditions, due to existing chloride salts of sodium, the chloride ion in the wastewater with high TDS is converted to chlorine which is further converted to hypochlorous acid in the aqueous solution, and the formed hypochlorite ions act as the main oxidizing agents in the pollutant degradation (Eqs. 5–7):¹⁸



According to Eqs. 8 and 9, at neutral pH, the removal efficiency was less than at acidic pH. This result can be attributed to the undesirable side reactions, such as oxidation of free chlorine to chlorate and perchlorate, formation of chlorate by chemical combination of hypochlorite and water, electrolysis of water and cathodic reactions involving loss of hypochlorite.²¹



Due to a nonsignificant difference between removal rates in the studied range of pH (p -value = 0.184 for pH values of 3 and 4, and 0.675 for pH values 4 and 5) and the feasibility issue of real industrial wastewater treatment, pH 5 was chosen for the remaining experiments.

Sonication has proved to be effective in enhancing the efficiency of EK oxidation for the degradation of real wastewaters.¹⁶ According to Fig. 2b, by changing the US power between 100 and 400 W, the COD removal increased from 11.7 % to 24 %, and the

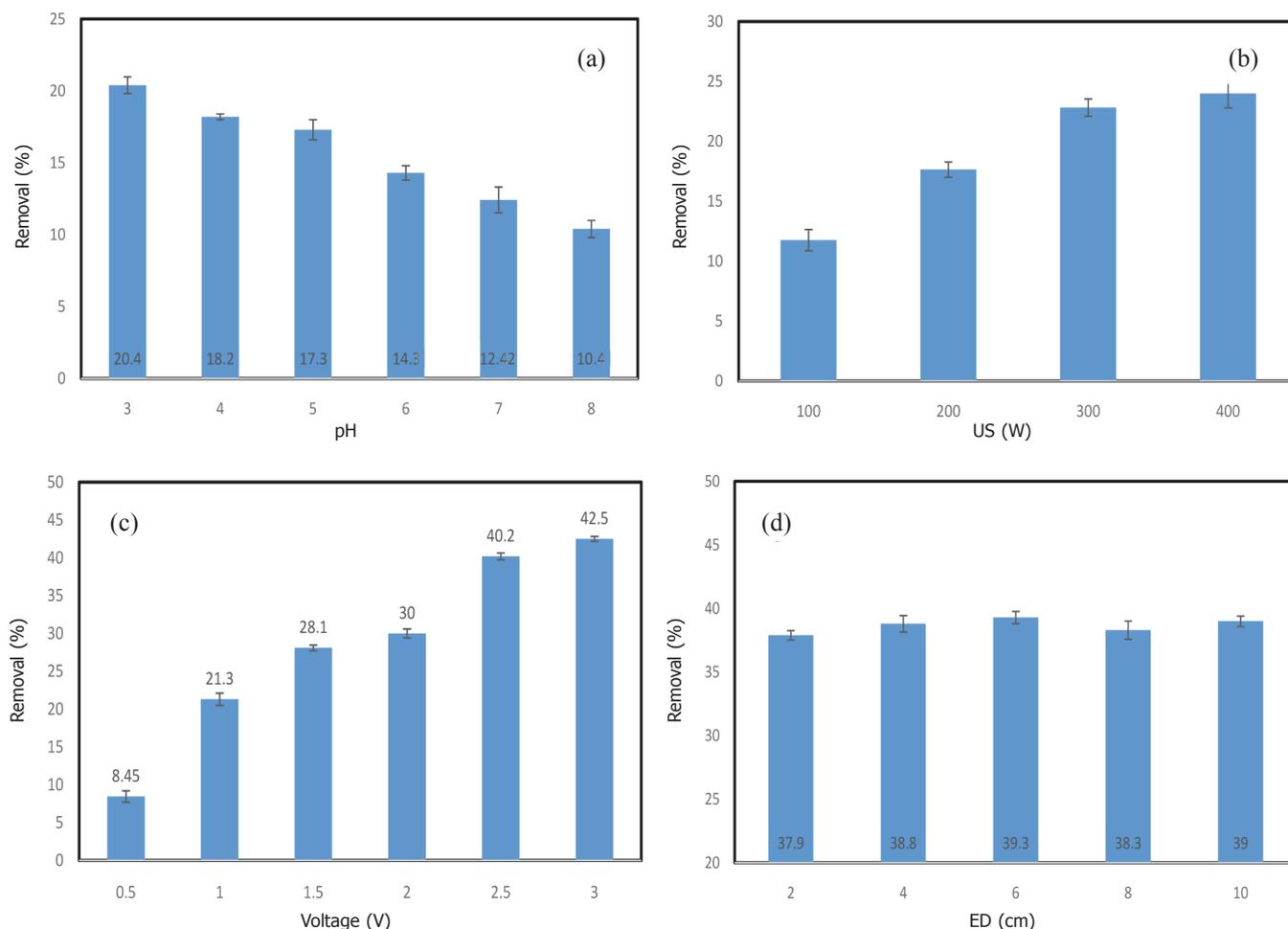
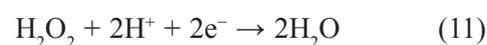
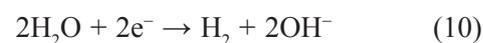


Fig. 2 – a) Effect of pH on COD removal of real saline oily wastewater (initial COD: 4100 mg L⁻¹, voltage: 1 V, electrode distance 8 cm, reaction time 2 h); b) Effect of sonication (pH: 5, COD: 4250 mg L⁻¹, voltage: 1 V, electrode distance 8 cm, reaction time 2 h), c) Effect of voltage (pH: 5, COD: 4020 mg L⁻¹, US level: 300 W, electrode distance 8 cm, reaction time 2 h); d) Effect of ED (pH: 5, COD: 4300 mg L⁻¹, US level: 300 W, voltage: 2.5 V, reaction time 2 h)

respectively, in which the observed COD removal for US levels of 300 W and 400 W showed nonsignificant difference (p -value > 0.05). Enhancement of the US power provides more energy for pulsation and the collapse of bubbles. Accordingly, higher cavitation bubbles and reactive radicals would be produced in solution.^{22,23} Along with US irradiation, a variety of oxidizing species (H₂O₂ and HO•) can be created in aqueous media because of cavitation phenomenon or water sonolysis, which causes further chemical reactions in different phases: internal cavity, interface boundary layer, and liquid bulk.²⁴ The reactions with HO• would occur in the interfacial boundary layer regions at normal temperature and pressure, as previously expressed in Eqs. (1–3).²⁵ The US power of 300 W was selected as the designed level, due to a nonsignificant difference between COD removal in 300 W and 400 W and less energy consumption.

Changes in applied voltage significantly influenced the COD removal in a way that, by increasing voltage, the removal was enhanced. In order to

determine the best technical and cost-effective level, a voltage range of 0.5–3 V was investigated under the operating conditions mentioned previously and the initial pH 5. The highest COD removal efficiencies of 40.2 % and 42.5 % were observed for voltages of 2.5 and 3 V, respectively (Fig. 2c). The enhancing effect of voltage can be attributed to the increasing rate of H₂O₂ production.¹⁴ An extra increase in voltage level, due to increased hydrogen gas production in the cathode, as well as increasing energy consumption can adversely affect the process efficiency. When the voltage is increased, the side reactions presented in Eqs. (10, 11) occur at the cathode, which leads to a higher generation of hydrogen gas.^{15,26}



Because of the lower power consumption and an insignificant difference (p -value > 0.05) in COD removal between voltages 2.5 and 3 V, the voltage

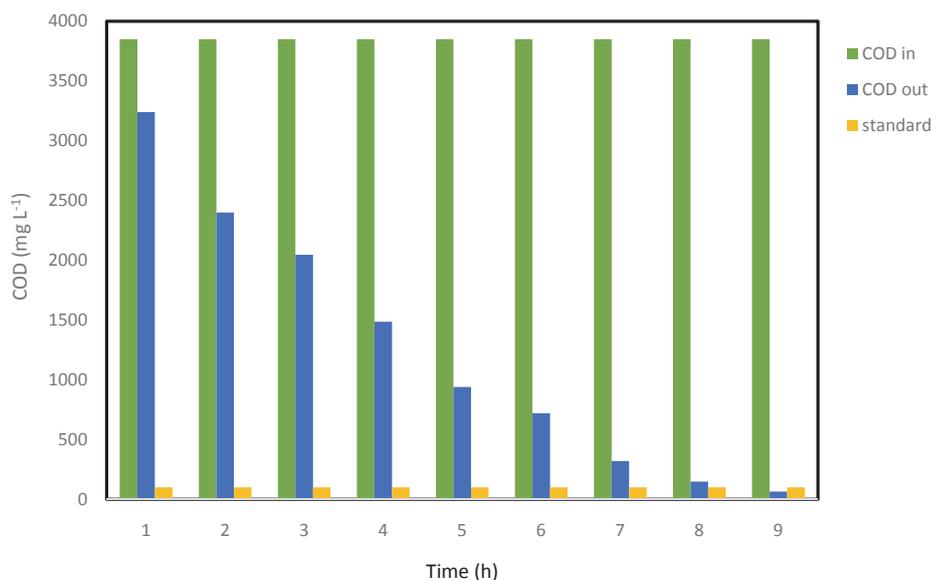


Fig. 3 – Effect of reaction time on COD removal of real saline oily wastewater (initial COD: 3850 mg L⁻¹, US: 300 W, voltage: 2.5 V, ED 8 cm, reaction time 9 h)

level of 2.5 V was selected for the remaining experiments.

Effect of ED on COD removal was also studied at four-electrode distances of 2, 4, 6, 8, and 10 cm under operating conditions including initial pH value of 5, voltage 2.5 V, US power 300 W during 2 h. Based on Fig. 1d, the COD removal efficiencies ranged between 37–39 % at EDs of 2, 4, 6, 8, and 10 cm, indicating no significant effect of ED on results.

Oxidation duration

Since obtaining the discharge standards to receiving waters was defined as a critical goal of the present work, improving the COD removal was attempted by increasing the reaction time and using predetermined operating conditions. Results showed the significant effect of contact time on COD removal with a sharp gradient up to 9 h, in which the COD removal of 98 % was observed for initial COD concentration of 3850 mg L⁻¹ (Fig. 3). Degradation of readily degradable and simple-structure compounds occurred at first, but mineralization and destruction of more resistant substances and intermediates required more reaction time of up to 9 h to obtain discharge standards.^{20,27}

Alternative processes and kinetic study

Enhancement effect of SEK process over EK alone and US alone was also studied and reaction rate constants were determined. As may be seen from Fig. 4, the COD removal rate was significantly higher for SEK (98 %) over EK (70 %) and the

US (12 %) processes. To determine the reaction rates of the SEK process, pseudo-first-order and pseudo-second-order kinetic models were evaluated under selected conditions using Eqs. (12 and 13):²⁸

$$\ln C_0/C_t = k_1 t \quad (12)$$

$$1/C_t - 1/C_0 = k_2 t \quad (13)$$

where, C_0 and C_t represent the concentrations of COD at the beginning and end of reaction in saline oily wastewater (mg L⁻¹), respectively, t is the reaction time (h), and k_1 (h⁻¹) and k_2 (L mg⁻¹ h⁻¹) are the corresponding rate constants. The reaction rate constant and correlation coefficient of the pseudo-first-order model for COD removal of saline oily wastewater using the SEK process was obtained by plotting $\ln(C_0/C_t)$ against time. According to Fig. 4, the kinetic parameters of the pseudo-first-order model were well-fitted with experimental data of COD removal with R^2 values of 0.93 with a corresponding reaction constant of 0.436 h⁻¹.

These higher removal rates were verified by a higher reaction rate constant of 0.43 h⁻¹ for SEK compared to the value of 0.13 h⁻¹ for EK and 0.01 h⁻¹ for the US.

Energy demand

The consumed electrical energy (E , kWh m⁻³) during the SEK oxidation is an important issue and was calculated using Eq. (14):²⁹

$$E = UI t / V \cdot 1000 \quad (14)$$

where U is the cell voltage (V), I is the average cell current (A), t is the reaction time (h), and V is the

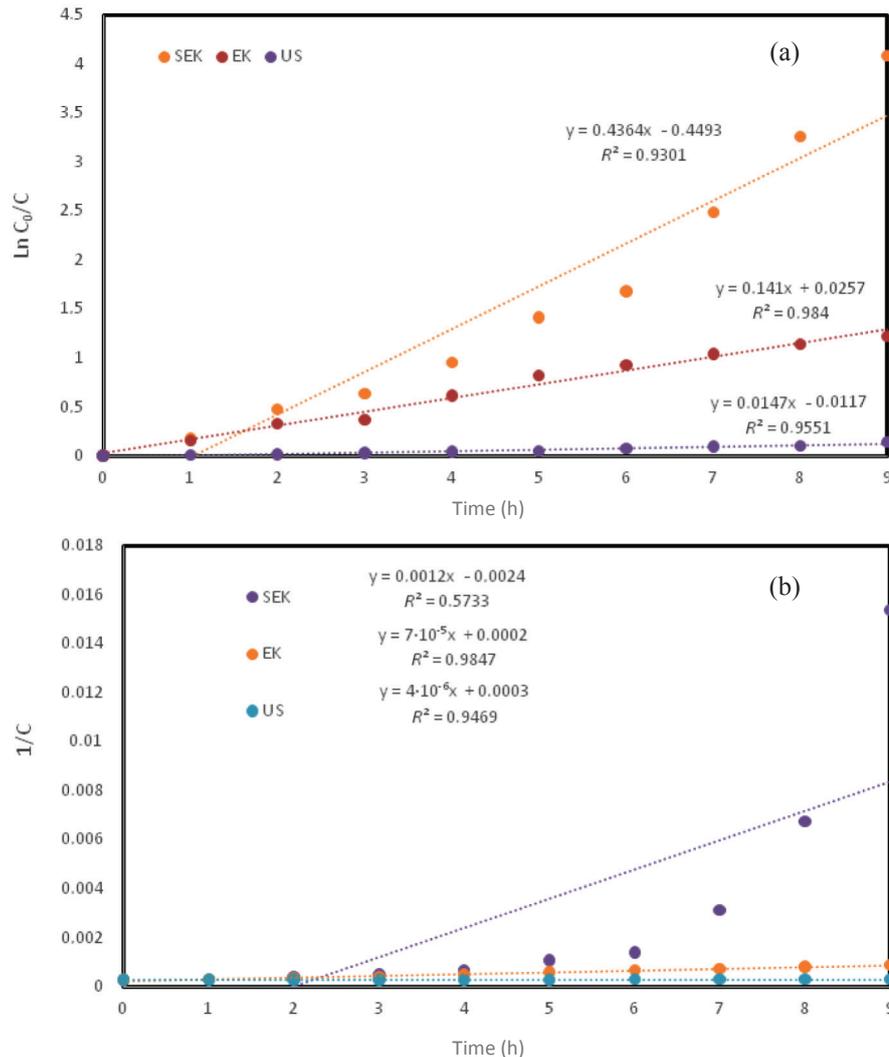


Fig. 4 – a) Pseudo-first-order kinetic, and b) Pseudo-second-order kinetic modeling of saline oily wastewater treatment using SEK, EK, and US irradiation processes at selected conditions (initial COD: 3850 mg L⁻¹, US: 300 W, voltage: 2.5 V, ED 8 cm, reaction time 9 h) compared to EK and US

treated volume (m³). Energy consumption was measured at selected conditions and initial COD concentration of 3350 mg L⁻¹. As was determined, the energy consumption of 12 kWh m⁻³ was obtained for SEK.

Biodegradability of effluent

Characterization of final effluent in terms of biodegradability is an important issue that indicates the toxicity status, as well as the possibility of integrating biological processes with complete treatment process, thus lowering the contribution of chemical-based technologies.³⁰ Under selected operating conditions, the effect of the SEK process on biodegradability improvement of effluent was evaluated using biodegradability assessment indices, including carbon oxidation state (COS), average oxidation state (AOS), and BOD₅/COD ratio. AOS and

COS values lie between -4 and 4 for methane and carbon dioxide as the most reduced and oxidized states of C, respectively. AOS and COS were calculated via Eqs. (15 and 16):^{31,32}

$$\text{AOS} = 4 - 1.5 \text{ COD/TOC} \quad (15)$$

$$\text{COS} = 4 - 1.5 \text{ COD/TOC}_0 \quad (16)$$

where, COD is chemical oxygen demand (mg L⁻¹), TOC is total organic carbon (mg L⁻¹) of the effluent after oxidation, and TOC₀ is initial TOC (mg L⁻¹) of wastewater sample. The final COD and TOC of final effluent were determined to be 65 mg L⁻¹ (98.3 %) and 43 mg L⁻¹ (98.4 %), respectively. Fig. 5 demonstrates that AOS and COS values varied from 1.6 to 1.73 and 3.95, respectively. This result indicated the removal of the majority of recalcitrant constituents or destruction of simpler structured forms.³³

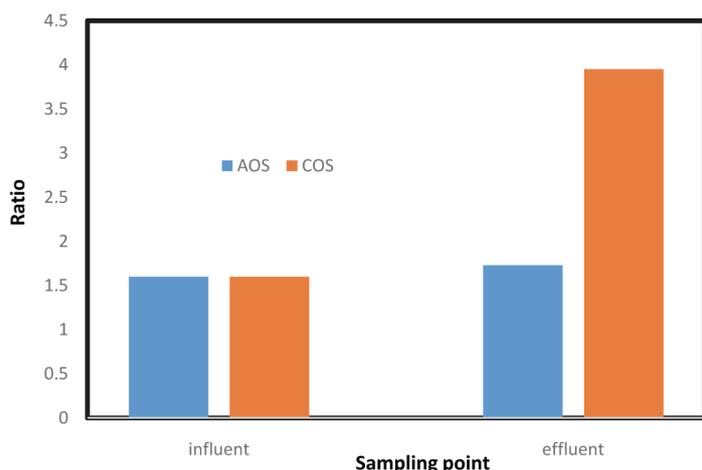


Fig. 5 – Variations of AOS and COS in the raw and treated sample of saline oily wastewater using SEK process

Toxicity assessment

Results of toxicity assessment are presented in Fig. 6. For the oxygen consumption rate inhibition (Fig. 6a), dehydrogenase activity inhibition (Fig. 6b), and growth rate inhibition methods (Fig. 6c), aside from raw wastewater, sonolysis alone showed the highest toxicity rate. The toxicity was highest for sonolysis, followed by EK, and SEK. The trends of toxicity evaluation are in accordance with removal rates. The highest removal rate of 98 % for SEK is in accordance with the lowest toxicity data (oxygen consumption rate inhibition: 12 %, dehydrogenase activity inhibition: 15 %, and growth rate inhibition: 14 %). From the toxicity response relationship, it may be seen that there was a good correlation between the oxygen consumption rate inhibition method, dehydrogenase activity inhibition method, and the growth rate inhibition method, in which the same trend of responses was observed for all studied alternatives. Lower toxicity effects in

SEK can be attributed to the enhancement of bio-availability. These results are in agreement with the literature.²⁸

Conclusion

An EK-based process enhanced by sonication was investigated for the treatment of a real saline oily wastewater containing different hydrocarbons with low biodegradability. Effect of operating variables, including initial pH, voltage, US power, electrode distance, and reaction time addition was studied to determine the most efficient conditions for COD removal. Results indicated that the process was pH-dependent, and the best results were observed in acidic pH conditions. In addition, by increasing the voltage, sonication power and reaction time improved the COD removal significantly through enhancement of the production rate of H_2O_2 , HOCl, and Cl_2 . Evaluation of removal results with conventional kinetic models demonstrated that COD removal by the SEK process followed pseudo-first-order kinetics. Increasing the AOS and COS indices of final effluent proved the enhancement of biodegradability characteristics of saline oily wastewater. The COD removal of around 98 % in reaction time of 9 h, as well as the energy consumption of 12 kWh m^{-3} , indicated that this technology is a reliable, efficient, promising, and cost-effective alternative for the treatment of saline oily wastewaters containing recalcitrant hydrocarbons, and supplemental pilot and full-scale studies are proposed to develop the technology and identify all aspects as a trading technology.

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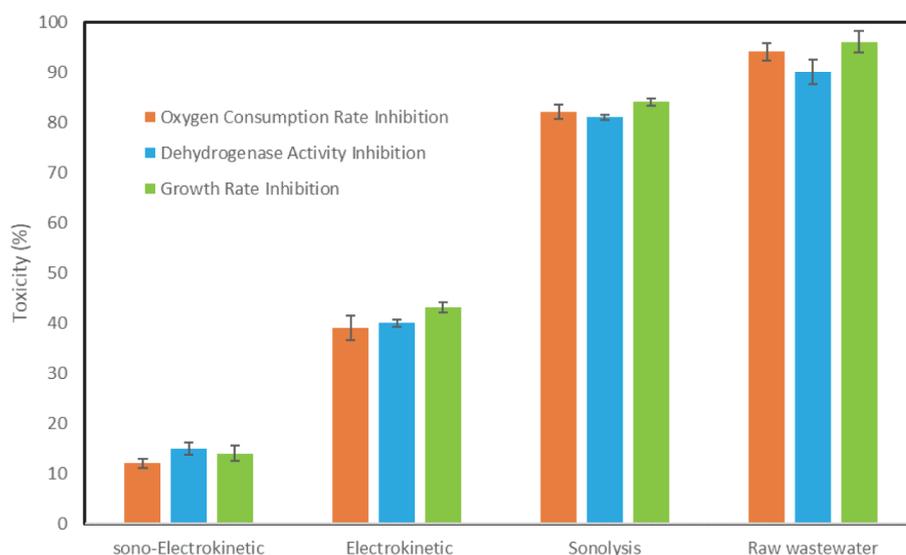


Fig. 6 – Results of toxicity assessment in different treatment alternatives, as well as in raw wastewater

Supplementary file 1

Oxygen Consumption Rate Inhibition Method

The oxygen consumption rate inhibition method refers to ISO 8192 (2007) (Diagne *et al.* 2007), and it has been improved appropriately. The bacterial consortium was formulated into an inoculum of 4 g L⁻¹. Formulated with nutrient substrate: peptone 16 g L⁻¹, beef extract 11 g L⁻¹, urea 3 g L⁻¹, NaCl 0.7 g L⁻¹, CaCl₂·2H₂O 0.4 g L⁻¹, MgSO₄·7H₂O 0.2 g L⁻¹, K₂HPO₄ 2.8 g L⁻¹, sodium acetate 90 mg L⁻¹. Add 1 mL of nutrient substrate, 1 mL of water, and 10 mL of sample to the aeration tube, mix and add 8 mL of bacterial inoculum, and aerate for 30 minutes. The rate of oxygen consumption was measured using an activated sludge respirator (Hach, Germany). The oxygen consumption rate inhibition is calculated using Eq. (A):

$$I = (D_0 - D_t)/D_0 \cdot 100 \% \quad (\text{A})$$

where I is the oxygen consumption rate inhibition rate; D_0 is the oxygen consumption rate of the blank control group; D_t is the oxygen consumption rate of the test group.

Nitrification Rate Inhibition Method

The nitrification rate inhibition method refers to ISO 9509 (2006) (Marselli *et al.* 2003), and it has been improved appropriately. The bacterial inoculum was formulated into an inoculum of 3 g L⁻¹, 5.04 g of sodium bicarbonate and 2.65 g of ammonium sulfate were dissolved in 1 L of water. N-allylthiourea with 1.16 g L⁻¹ was prepared. 10 mL culture medium, 50 mL bacterial inoculant and 40 mL sample were added to the conical flask. No toxic substances were added to the blank group and the control group, and 1 mL ATU solution was added to the control group. All other substances added were the same. The sealed samples were cultured at a rotation speed of 130 rpm at 22 °C for 4 hours in a constant-temperature incubator (IKA KS 4000 ic control, Germany). After culture, the concentration of nitrite and nitrate were determined according to standard methods for examination of water and wastewater (Eaton *et al.*). The nitrification rate of bacterial inoculum can be calculated using Eq. (B):

$$R_N = (\gamma_t - \gamma_b) / (\gamma_{MLSS} \cdot 4) \quad (\text{B})$$

where R_N is the nitric acid rate of bacterial inoculum; γ_b is the mixed concentration of nitrite and nitrate of bacterial inoculum in the blank group after 4 hours, mg L⁻¹, γ_t is the mixed concentration of nitrate and nitrite in the inhibition group with ATU after 4 hours, mg L⁻¹, γ_{MLSS} is the concentration of bacterial inoculum in the mixed solution in the test vessel, g L⁻¹.

The formula for calculating the nitrification inhibition rate is as follows Eq. (C):

$$I_N = (\gamma_c - \gamma_t) / (\gamma_c - \gamma_b) \cdot 100 \% \quad (\text{C})$$

where I_N is the nitrification inhibition rate; γ_c is the concentration of nitrite and nitrate in the blank control group after culture, mg L⁻¹, γ_t is the concentration of nitrite and nitrate in the test group containing toxic substances after culture, mg L⁻¹, γ_b is the concentration of nitrite and nitrate in the control group containing ATU inhibitor after culture.

Growth Rate Inhibition Method

The growth rate inhibition method refers to ISO 15522 (1999) (Soltani *et al.* 2016a), and it has been improved appropriately. The bacterial inoculum was precipitated for 15 min, and the supernatant was taken for inoculation. The operating environment was sterilized before testing. Formulation solution A: 8.5 g of anhydrous potassium dihydrogen phosphate, 21.75 g of dibasic potassium hydrogen phosphate, 33.4 g of disodium hydrogen phosphate dihydrate, and 0.5 g of ammonium chloride in 1 L of water were dissolved. Solution B: 22.5 g L⁻¹ magnesium sulfate solution. Preparation solution C: 36.4 g L⁻¹ calcium chloride solution. Formulation solution D: 0.25 g L⁻¹ solution of iron chloride hexahydrate. Formulation solution E: Dissolve 50 mg of boric acid, 50 mg of cobalt chloride hexahydrate, 15 mg of manganese sulfate monohydrate, 15 mg of disodium molybdate, 10 mg of nickel chloride, 50 mg of zinc sulfate heptahydrate in 1 L of water. Formulation solution F: 80 g of beef extract and peptone mixture and 60 g of sodium acetate and 1 liter of water were dissolved. The culture solution was prepared by adding 800 mL of water, adding 10 mL of solution A, taking 1 mL of each solution from B to E, adding 25 mL of solution F, and formulating 1 L in a volumetric flask. According to the test method, after pre-culture and main culture, 20 mL of the main culture bacterial inoculum was added to the Erlenmeyer flask, and 5 mL of the sample was added. After shaking, 3.5 mL of the solution was taken, and the sealing film was placed thereon, and cultured at a rotation speed of 150 rpm at 22 °C. The absorbance value of the removed solution was measured. The test was taken once every hour, and then the culture was continued according to the previous procedure. After the fifth sampling, the test was completed. The growth rate inhibition rate was calculated according to Eq. (D):

$$I = (V_0 - V_1) / V_0 \cdot 100 \% \quad (\text{D})$$

where I is the growth rate inhibition rate; V_0 is the microbial growth rate of the blank control group; V_1 is the microbial growth rate of the test group to which the toxic substance is added.

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