Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2020.26431

207 (2020) 361–372
December

Improvement on electrochemical inactivation of *Escherichia coli* and *Clostridium perfringens* by assisted alum nanocrystallites approach: parametric and cost evaluation

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Received 17 February 2020; Accepted 6 August 2020

ABSTRACT

Electrocoagulation (EC) is an electrochemical method in water treatment. Nowadays, electrochemical disinfections have created great interest in water treatment as an alternative for conventional disinfection. In this study, the effects of electrochemical inactivation with monopolar electrodes on *Escherichia coli* and *Clostridium perfringens* bacteria removal from potable water by the EC process were investigated. In addition, the effects of initial pH, reaction time, current density, as well as inter-electrode distance and conductivity on abatement of selected bacteria along with operating costs have been studied. The structure and surface morphology of the alum nanocrystallites were investigated by X-ray fluorescence, N_2 adsorption–desorption, transmission electron microscopy, powder X-ray diffraction, and field emission-scanning electron microscopy. The experiments were performed in a batch reactor. According to the results, the optimum condition for removal efficiency of 100% *E. coli* and *C. perfringens* with an 0.6 USD/m^3 operation cost for the treated aqueous solution were as follows: reaction time = 25 min, initial pH = 7, inter-electrode distance = 2 cm, current density = 33.3 A/m^2, electrical conductivity = 0.75 mS/cm, and initial bacteria count = 10^4 CFU/mL. Findings indicated that increasing pH from 7 to 9 can significantly decrease the removal efficiency for *E. coli* and *C. perfringens* from 100% to 74% and 71%, respectively. Increasing reaction time and current density decreased both strains significantly. Inter electrode distance had an intangible effect on strains abatement. Field Emission-Scanning Electron Microscopy analysis revealed morphological variations and the mechanism through which the killing and trapping of the bacteria by alum nanocrystallites occur. The present method is able to reduce the *E. coli* and *C. perfringens* count in potable water, which meets the drinking water standards according to WHO guidelines.

Keywords: Electrocoagulation; *Escherichia coli* removal; *Clostridium perfringens* removal; Alum nanocrystallites; Operating cost

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1. Introduction

Disinfection is well-known as the most important step in providing safe potable water [1,2], which typically includes multibarrier water treatment processes categorized as physical processes (settlement, sand, and membrane filtration) [3], chemical (chlorination, ozonation) [4–6] as well as physico-chemical processes (coagulation/flocculation) [7,8]. The disinfection process is considered to have two main purposes which are as follows: primary disinfection occurs in raw water supply and abate or inactivate microbiological contaminants [9], and the residual is provided in the distribution network [10]. The addition of chlorine and/or chlorine by-products is one of the most popular and commonly used chemical methods for disinfection which presents both primary and residual disinfection [11]. Although chlorine as a disinfectant for water treatment has great advantages, however, consumer-related issues and regulatory pressure on water supply companies are its several disadvantages and include unfavorable taste and odor instigated by using chlorine in potable water, being ineffective against resistant pathogens such as *Cryptosporidium parvum*, when used alone [12], and the production of toxic disinfection by-products (DBPs) such as chloroform which are a major health concern.

Compared to conventional treatment methods, EC process has been attracted by the researcher because of great abatement performance, better environmental compatibility, simplicity, reliability, versatility, cost efficiency, and the possibility of complete process automation, which provides both primary and residual disinfection [13,14]. The advantages of electrochemical disinfection such as its effect on inactivating various microorganisms including viruses, bacteria, algae, and also larger species such as *Euglena*, have made it to be attractive compared to other available methods. This process has been developed as a robust, versatile, cost-effective, and energy-efficient alternative for disinfection [15]. The electrochemical disinfection process includes forcing water by a disinfector which is equipped with anode and cathode electrodes on which current is charged [8]. EC is processed based on applying electrodes of anode such as aluminum (Al) and iron in order to generate metal ions to supply ions into the treated water and can be used to coagulate in an aqueous phase and also provide the active sites suitable for more adsorption of contaminants [16].

Apart from adsorption, important phenomena that also play a role in the abatement of pollutants in EC process are sweep coagulation, coprecipitation, bridge coagulation, etc. [17].

The electrochemical reactions when Al is considered as an electrode are as follows [14]:

Reaction during the anode section:

$$Al \rightarrow Al^{3+} + 3e^{-} \quad (1)$$

Reaction during the cathode section:

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

Reaction takes place in aqueous phase:

$$Al^{+3} + 3OH^{-} \rightarrow Al(OH)_3 \quad (3)$$

Also, in EC process Al and OH^- ions produced by reaction (1) and (2), react to produce monomeric kinds such as $Al(OH)^{2+}$, $Al_2(OH)_2^{4+}$, $Al(OH)_4^-$, and polymeric moieties such as $Al_8(OH)_{20}^{4+}$, $Al_7(OH)_{17}^{4+}$, $Al_6(OH)_{15}^{3+}$ [18]. These species further react and lead to amorphous $Al(OH)_3$ based on ligand sedimentation kinetics [19]. Electrochemical disinfection has been discussed since 1950s and then its great effectiveness has been demonstrated in several liquid media [8]. Free radicals, including $O_2^{\bullet-}$ and $^{\bullet}OH$, which are generated in the electrochemical reactions, have recently gained considerable attention, but the debate on the possible mechanism of electrochemical bactericidal property is still open [20,21].

Clostridium perfringens is one of the major concerns in water treatment researches, mainly because it forms spores and survives in water for much longer than coliform and also it has a low sensitivity towards common disinfectants such as chlorine and chlorine [22–24]. So, recently, *C. perfringens* and *Escherichia coli* have been reported as a suitable indicator for the existence of pathogens of fecal source in surface waters and a biological index of sterilization efficiency in water treatment plants [22,25,26]. Considering the above-mentioned points, *C. perfringens* has been selected in this research. In this study, *E. coli* and *C. perfringens* cells were employed as a clear application example of electrodisinfection by cathodes (stainless steel-ST) and anodes (aluminum-Al) in parallel connections at monopolar electrodes. The batch experiments were performed in a constant initial amount of *E. coli* and *C. perfringens* count in to examine the factors affecting the process such as (reaction time, current density (CD), initial pH, inter-electrode distance (IED), and conductive) on the percentage removal of *E. coli* and *C. perfringens*. Moreover, the operating cost of the selected treatment was evaluated.

Initial count of *E. coli* and *C. perfringens* plays an important role in removal efficiency. Therefore, in the present research the role of initial count in removal efficiency were investigated.

2. Materials and methods

2.1. Chemicals, water substrates, and bacterial strains

All applied chemicals were analytical grade and utilized without further purification. *E. coli* (ATCC25922) and *C. perfringens* (ATCC 10543) were provided by Pasteur Institute of Iran. All culture media and chemicals were purchased from Merck Company. Physicochemical features of the solutions before EC process are presented in Table 1.

2.2. Preparation of bacterial strains

The *E. coli* strain was cultured in Nutrient Broth medium for 24 h at 37°C (aerobic conditions). With a sterile inoculating loop a loopful of the suspension was streaked onto MacConkey agar and further incubated at 37°C for 48 h [27]. Thioglycollate (FTG) medium was used for *C. perfringens* strain growth. The culture was incubated in anaerobic condition for 48 h at 37°C. Then, a loopful of bacterial suspension was sub-cultured onto SPS agar and incubated in anaerobic condition at 37°C for an additional

Table 1
Physicochemical characteristics of the investigated solutions

Conductivity (mS/cm)	0.75
Total hardness (mg/L Ca, Mg)	277.55
Hardness (mg/L Ca)	131.775
Total alkalinity	225.5
Na (mg/L)	173.38
K (mg/L)	19.07
Nitrate (mg/L)	6.01
Sulfate (mg/L)	67.02
Chloride (mg/L)	96.23
Turbidity (NTU)	0.46
TDS (mg/L)	576.5
pH	7.5
Temperature (°C)	18

48 h [28]. A 1.5 L suspension of tested bacteria in sterile drinking water with 10^3, 10^4, and 10^5 CFU/mL were prepared by adding 10, 100, and 1,000 μL of bacterial culture in peptone broth (1.5×10^8 CFU/mL), respectively, in the room temperature.

2.3. Instrument setup

The batch experiments of EC were performed in a Plexiglas reactor (135 mm × 105 mm × 170 mm) with 1.5 L volume. Two Al anodes and two ST cathodes (100 mm × 40 mm × 2 mm) were connected to a digital DC power supply (Fe and Al purity: 99.5%) (Dazheng DC Power supply PS-302D) with 0–25 V and 0–10 A. ST and Al electrodes were connected to negative and positive poles of DC power, respectively. The experimental setup has been shown in Fig. 1. The total effective electrode area was 240 cm^2 and the constant current was adjusted according to a desirable value (8.3–33.3 A/m^2). The CD was computed by the equation as follows:

$$CD = \frac{I}{6 \times S} \tag{4}$$

where I indicates electrolysis current (A), and S indicates an area of the electrode (m^2).

2.4. Experimental procedure

Due to ensure great surface reproducibility, before each test, the Al or ST electrodes were handmade furbished utilizing sandpaper, dehydrated in acetone, washed with ultra-pured water, and then dipped in watery nitric acid, washed again with deionized water, after washing, electrodes were dehumidified at 105°C and afterwards weighted before immersion in the solution. Before beginning each test, the experimental equipment was disinfected for 30 min with warm water flow at the temperature of 70°C–88°C. The effects of initial pH, CD, reaction time, IED, and conductivity were investigated at various conditions as represented in Table 2. In each test, 1.5 L of a potable water (temperature, 25°C) sample containing bacterial counts (10^3–10^5 CFU/mL) was placed into the EC reactor (Fig. 1). The cathodes and anodes were linked to the negative and positive outlets of a DC power supply, respectively.

At the end of EC, about 5 mL of samples were derived from the liquid phases for chemical and bacterial characterization.

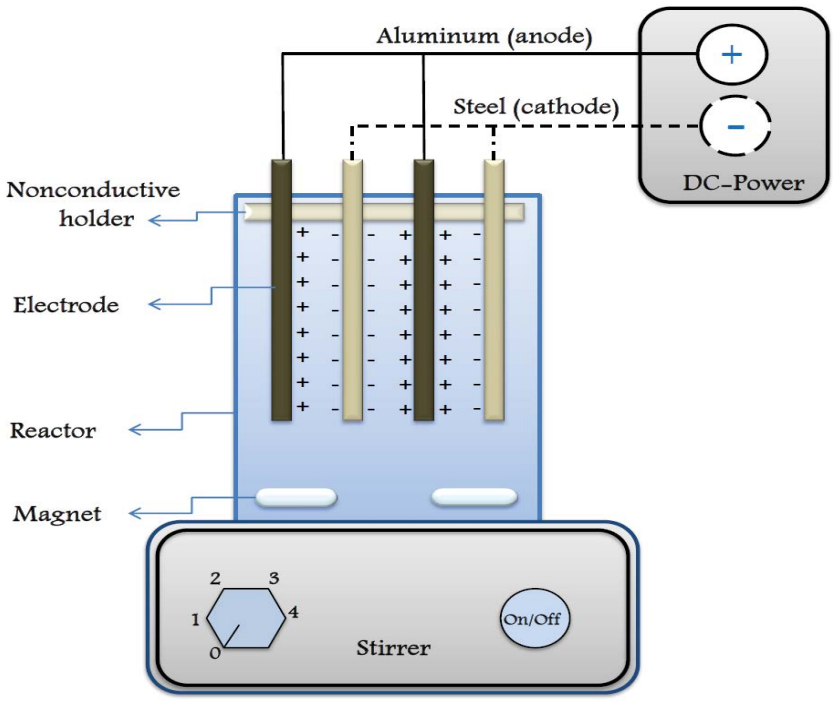

Fig. 1. Schematic diagram of the pilot reactor with cathodes and anodes parallel connections at monopolar electrodes.

Table 2
Range of operating factors for *E. coli* and *C. perfringens* abatement by EC

Type of experiments	Primary pH	Current density (mA/cm^2)	Reaction time (min)	IED (cm)	Electrical conductivity (mS/cm)	Initial *E. coli* count (CFU/mL)	Initial *C. perfringens* count (CFU/mL)
Effect of initial pH	7–9	3.3	25	2	0.75	10^4	10^4
Effect of current density (mA/cm^2)	7	0.83- 3.3	0–25	2	0.75	10^4	10^4
Effect of run time (min)	7	3.3	25	2	0.75	10^4	10^4
Effect of IED (cm)	7	3.3	25	1–3	0.75	10^4	10^4
Electrical conductivity	7	3.3	25	2	0.75	10^4	10^4
Effect of *E. coli* count (CFU/mL)	7	3.3	25	2	0.75	10^3–10^5	–
Effect of *C. perfringens* count (CFU/mL)	7	3.3	25	2	0.75	–	10^3–10^5

The pH, temperature, and conductivity before and after the EC were tested.

Conductivity, pH, and temperature of solutions were measured via a conductivity meter (Mettler Toledo 7100e), a pH meter (Mettler Toledo 2050e), and a thermometer (Mettler Toledo 51343310). pH of the solutions was adjusted by adding either 1N NaOH or 1 N HCL. The conductivity was increased by adding NaCl. Samples were magnetically stirred during the EC process at the speed of 300 rpm (HP100) to decrease the mass transport over the potential of the EC reactor. After the EC process, samples were stirred with an overhead stirrer at the 50 rpm for 10 min to aid flocculation. Settling time after flocculation was 30 min. The Feasibility of each purification process for usage on a large-scale can be mainly affected by the purification process cost. The cost of EC process includes a material cost (mainly chemical and electrodes), consumption cost (mainly electrical energy) cost, as well as maintenance, worker, and other costs.

The latter cost items are independent of the kind of the electrode material.

Therefore, because the major cost is related to energy, electrode materials, and chemical, hence these three items were considered as operating costs according to Eq. (5) [13,29].

$$\text{Operating cost} = a \times C_{\text{Electrode}} + b \times C_{\text{Energy}} + c \times C_{\text{chemicals}} \quad (5)$$

where, $C_{\text{Electrode}}$ (kg Al/m^3), C_{Energy} (kwh/m^3), and $C_{\text{chemicals}}$ (kg chemicals/m^3) are utilization of amounts for the removal of *E. coli* and *C. perfringens* water contaminants.

Unit prices, *a*, *b*, and *c* have given from the Iranian market in the year 2015, are as follows:

- *a* is whole sale electrode material price = 2 USD/kg Al (http://www.iraninternationalmagazine.com/issue_74/textsp/the%20most%20advance%20aluminum%20production%20complex%20in%20iran.htm)
- *b* is industrial electricity price = 0.027 USD/kwh^{-1} (https://en.trend.az/iran/2247258.html)
- *c* is prices of NaOH and H_2SO_4 as 1.3 and 0.5 USD/kg, respectively.

The electrical energy consumption (EEC) in EC process was achieved by Eq. (6).

$$E = \frac{IUT}{V} \quad (6)$$

where *E* is electrical consuming energy (kWh/m^3), *I* is current (A), *U* is reactor voltage (V), *V* is volume (m^3), and *T* is reaction time (h). Electrode material cost was obtained by subtracting the primary and final mass of anode before and after EC process.

2.5. Analytical procedure

Five milliliters of samples were taken from running EC reactors in five min (5–25 min) time intervals. The samples were kept on bench top at room temperature for 30 min without agitation. One-hundred microliters of supernatant from *E. coli* and *C. perfringens* samples were streaked on MacConkey agar and SPS medium, respectively. MacConkey agar plates were incubated for 48 h at 37°C in aerobic condition and SPS plates were incubated at 37°C in anaerobic condition for 48 h. Then the colonies were counted (CFU/mL) to estimate the percentage of bacteria removal using Eq. (7). Where C_{in} indicates initial count (CFU/mL), C_{out} indicates the final count (CFU/mL). As a control, the count of bacteria in the suspension upstream of the EC unit was also specified.

$$E\% = \left(\frac{C_{\text{in}} - C_{\text{out}}}{C_{\text{in}}}\right) \times 100 \quad (7)$$

All chemical and microbial experiments were performed based on the standard methods suggested by APHA [30], except where noted.

2.6. Purification of alum nanocrystallites

The decanted flocs were separated at the end of the tests and afterward dried at 25°C in a well-covered container for a period of up to 72 h. To purify the alum NCs of electrochemically produced sludge it is necessary to recognize the chemical composition of the sludge. Hence,

XRF analysis (Philips PW 1410, Netherlands) was utilized to determine the components of the EC sludge. The experimental procedure for the preparation of alum NCs is described elsewhere [31].

2.7. Alum nanocrystallites characterization

The alum NCs were separated at the end of the purification process and analyzed with various techniques. Brunauer–Emmett–Teller (BET) surface areas of the alum NCs were determined by N_2 adsorption/desorption on a BELSORP-mini II analyzer (BELSORP, Japan) at 196°C. Samples were degassed for 2 h at 120°C before analysis. Transmission electron microscopy (TEM) was performed on a (Philips CM 30, Netherlands) operating at an accelerating voltage of 200 kV. Samples sonication was carried out to break down their alum NCs homogenously. Then, the TEM samples were prepared by drying a small drop containing alum NCs on holey carbon TEM grid sand at room temperature for 2 h in air.

FE-SEM was performed on a HITACHI S-4160FESEM operated at a 20 kV accelerating voltage. A small drop containing alum NCs was spread onto a silicon wafer and was dried almost completely in air at room temperature for 2 h. The wafer then was transferred onto FE-SEM conductive tapes and was sputter coated with a thin layer of gold before analysis. XRD spectroscopy (Philips PW1730, Netherlands) using CuKα radiation with tube voltage of 40 Kv with a step size of 0.05 and a step duration of 1 s in a step-scanning mode, from 2θ values of 10°–80°.

2.8. Data analysis

Statistical studies of data was created out by analysis of variance (one-way ANOVA). Statistical magnitude was supposed at $P < 0.01$. All measurements were performed three times in two replicates and the mean amounts are represented.

3. Results and discussion

3.1. Effect of EC on solution temperature

Current intensity determines the coagulant doses and also produces H_2, H_2O_2, O_3, and other molecular and ionic elements [9,32]. It changes the solution temperature [33]. Temperature rise during EC due to Joule effect [34]. Although EC is a non-thermal technology, depend on sample composition and process conditions. The temperature increased during EC treatment. In general, an increase in the intensity of this parameter would lead to enhanced microbial inactivation [33].

To control the effect of temperature on EC process, an initial study was done to choose the conductivity parameter. As can be seen in Fig. 2, the temperature increased as solution conductivity and reaction time increased. In a constant voltage (ΔV), current (I) of electrolysis depends on the resistance (R) of the solution between electrodes ($I = \Delta V/R = \Delta VG$). Increasing the solution conductivity increases the electrical current that consequently promotes the joule effect (RI^2) following by temperature increasing [35]. Most pathogenic microorganisms are capable to be viable at 10°C–45°C temperatures range [36]. The solution temperature was controlled at a temperature ≤25°C via selecting the less conductivity solutions (0.75 mS/cm).

3.2. Effects of experimental factors on bacterial abatement

3.2.1. Effect of inter electrode distance

The effect of IED between 1 and 3 cm was investigated. It is observed that, with increasing the IED from 1 to 2 cm, *E. coli* and *C. perfringens* removal efficiency increased from 96% and 94% to 100%, respectively, and reduces afterward from 2 to 3 cm. The very small IED (1 cm) causes difficulty in circulation of the solution among the electrodes, therefore abatement is less, and its enhancements slowly by increasing IED up to 2 cm. Once the IED increases, the resistance between electrodes (two electrodes) increases, and therefore, the current of electrical decreases. Therefore, the abatement of both *E. coli* and *C. perfringens* decreased with increasing IED from 2 to 3 cm. Therefore, the voltage must be amplified to obtain a certain CD [37]. By increasing the electrode distance, we have less interaction between *E. coli* and *C. perfringens* with the hydroxyl polymersis due to less local concentration and electrostatic attraction [13]. Also, Fig. 3a showed the effect of IED on the operating cost. Operating cost increased with increasing IED. When IED increased, the voltage across the electrode increased. Therefore, the power utilization increased to obtain the demanded CD.

3.2.2. Effect of solution conductivity

Electrical conductivity of the solution is a crucial factor in EC. For electrical current to pass through solutions, it must be quite large (about 1 mS/cm) [36]. NaCl is commonly utilized to increase the electrical conductivity of the solution [29]. In other words, ion concentration in the solution is increased with an increase in salt concentration, and therefore the resistance among the electrodes is reduced. Also, an increase in salt concentration at a steady current density, reduces its cell voltage, and thus reduces power consumption in electrolytic cells [38]. As Fig. 2 shows, the temperature of solution in the EC process can be handled with solution conductivity. As such, the temperature increases with increasing the solution conductivity, and

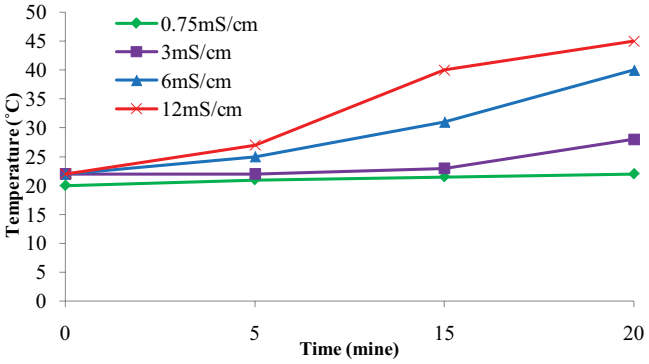

Fig. 2. Effect of duration and solution conductivity on the solution temperature.

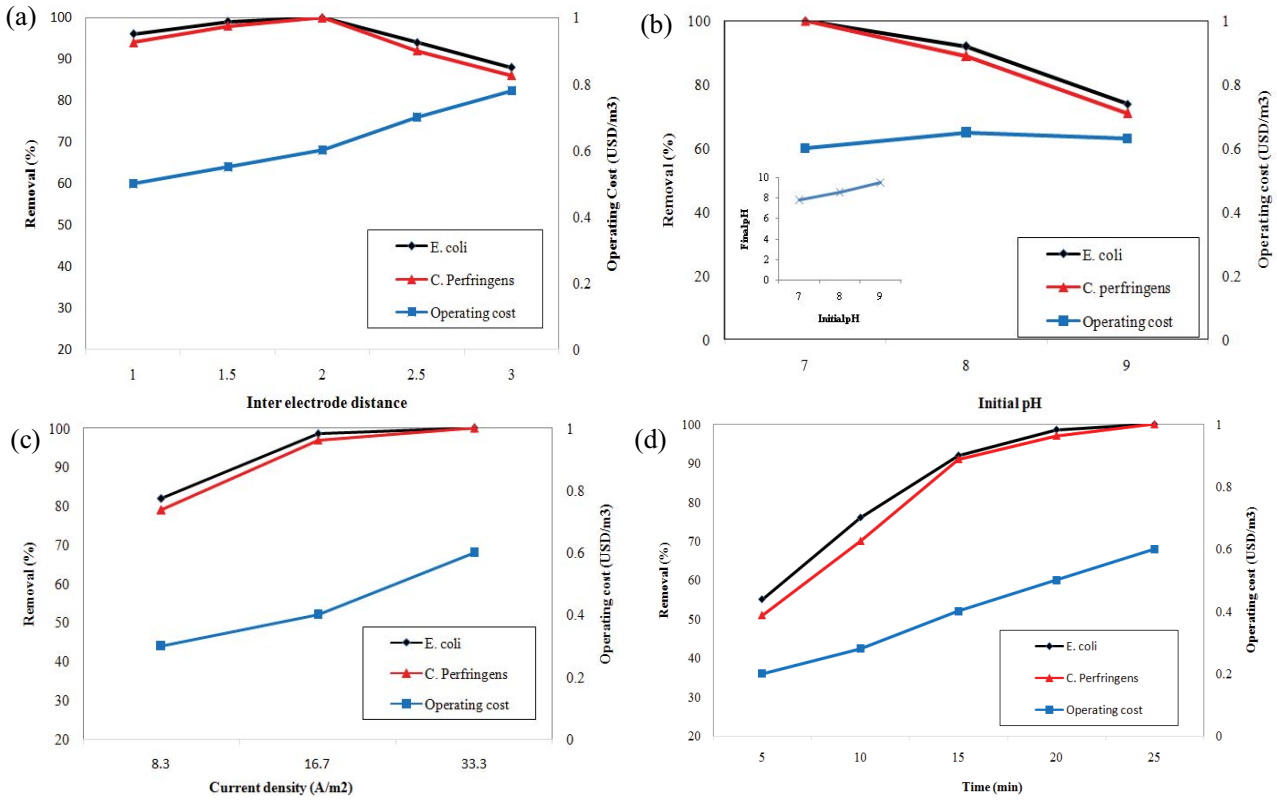

Fig. 3. Effect of IED (a), initial pH (b), current density (c), and reaction time (d) on removal of *E. coli* and *C. perfringens*, operating cost.

finally can interfere in kinetic modifications. As mentioned above, the temperature of the solution was controlled (less than 25°C) by selecting the solutions with less conductivity (0.75 mS/cm). The removal efficiencies of *E. coli* and *C. perfringens* in conditions such as 2 cm IED and solution conductivity 0.75 mS/cm was 100% (Fig. 3a).

3.2.3. Effect of initial pH

The pH plays a key role in the performance of EC [34,39]. It will determine the ionic characteristic of the metal hydroxides in solution and hence the bacteria abatement mechanism is directly affected by pH. To survey the effect of this factor on the treatment process, the experiments were performed according to the parameters mentioned in Table 2. Fig. 3b shows the effects of initial pH on removal efficiency of *E. coli* and *C. perfringens*, in addition it displays the plot of the initial and final pH. It is indicated from the figure that, the pH of solution increased during the reaction. This finding is in agreement with another current report [13] which concluded that this variation is dependent upon the initial pH and type of electrode. Enhancement in pH can be illustrated through formation of OH^- ions and H_2 gas at the cathode [38]. The removal efficiency of *E. coli* and *C. perfringens*, for the initial bacteria count of 10^4 CFU/mL, reduction at various pH has been shown in Fig. 3b. It is clear that the pH of initial has an impressive effect on the bacteria abatement efficiency. When the initial pH increased from 7 to 9, the removal efficiencies of *E. coli* and *C. perfringens* significantly reduced from 100% to 74% and 71%, respectively. Lower abatement efficiency at pH 9.0 is due to the predominance of an mononuclear anionic specie $Al(OH)_4^-$ under alkaline conditions, which cannot affect positively on electrolysis process [18,40].

Bacteria abatements are due to particles trapping in the flocs. However, the dissolution of Al released bacteria in the solution. Furthermore, increases in chemical reactions at pH 7 lead to a maximum electron transfer between bacteria and electrodes and decrease the electrode fouling [41,42]. In accordance with the results, abatement efficiency at pH 7 was the highest. Therefore, in the current study, pH 7 was considered as an optimum condition. Operating cost at 33.3 A/m^2 CD for different pH (7, 8, and 9) was almost identical and equal to 0.6 USD/m^3 (Fig. 3b).

3.2.4. Effect of current density

CD is the main factor in all EC processes. CD specifies the value of the generated coagulant, bubble generation, size, and enhancement of flocs which highly influence the abatement efficiency of EC [32,43].

The amount of substance dissolved is directly proportional to the quantity of electric current passed in solution during EC according to Faraday's law (Eq. (8)) [14].

$$W = \left(\frac{ITM}{ZFV}\right) \tag{8}$$

where W indicates metal dissolving Al (g), I indicates electrolysis current (A), T indicates reaction time (s), M

indicates Al molecular weight (27 g/mol), Z indicates number of electrons involved in the oxidation of Al (Z = 3), F indicates Faraday constant (96,500 Cmo/L), and V indicates volume (m^3). According to Eq. (8), the more current passes via process, the more would be the value of generated coagulant and bubble, which enhancement mixing rate inside EC process.

To enhance the CD would speed up the release rate of Al^{3+} and OH^- ions, which further assistant the accelerating of abatement efficiency. To find out the effect of CD on operating cost, abatement of *E. coli* and *C. perfringens*, were evaluated at three current densities (8.3, 16.7, and 33.3 A/m^2). The effect of CD on operating cost and removal efficiency of selected bacteria has been shown in Fig. 3c.

It can be seen from Fig. 3c that at CD 8.3 A/m^2, the operating cost, removal percentage of *E. coli* and *C. perfringens* are found 0.3 USD/m^3, 82% and 79%, respectively.

With increasing CD, operating cost and abatement percentage of *E. coli* and *C. perfringens*, have been increased.

At CD 33.3 A/m^2, percentage abatement of *E. coli* and *C. perfringens* reached to 100%, which meets the drinking water standards according to WHO guidelines [22]. This result is in good agreement with those found in the study of Ghernaout and Ghernaout [44] and Wei et al. [45], who found that the abatement efficiency of bacteria by EC increased with the increase of CD. This result is due to the fact that an increase in CD, increased the production of OH^- and Al^{3+} ions at cathode and anode, respectively, and their combination in the form of $Al(OH)_3$ when high CD is applied. Subsequently, it increases the production rate of flocs and hence enhances the bacterial abatement efficiency. These flocs will trap the bacteria and increase the bacteria abatement efficiency.

However, higher CD causes an elevated dissolution rate of Al anode which may result in more sludge production and therefore increase the cost of the treatment process. The WHO has proposed the use of *E. coli* and *C. perfringens* as the indicator organisms for potable water, which no one must be detected in 100 mL water. Although the cost of treatment in CD equal to 33.3A/m^2 (0.6 USD/m^3) compared to densities 8.3 (0.3 USD/m^3) and 16.7 A/m^2 (0.4 USD/m^3) is much more, but in regard to abatement efficiency values for *E. coli* and *C. perfringens* (100%), it is in a costly optimum condition for the process. Given this cause, all further tests were performed at CD of 33.3 A/m^2.

3.2.5. Effect of reaction time

The effect of reaction time on operating cost and removal efficiency of selected bacteria has been shown in Fig. 3d. The removal efficiency for both bacteria increased with increasing reaction time. It could be seen from Fig. 3d that during the first 5 min, operating cost, percentage abatement of *E. coli*, and *C. perfringens* are found to be 0.2 USD/m^3, 55% and 51%, respectively. At run time 25 min, percentage abatement of *E. coli* and *C. perfringens* reached 100%, which comes to the safe potable water according to WHO guidelines.

The increase in time is accompanied by more accumulation of coagulants inside the reactor, which might be the reason of high bacteria abatement. According to Faraday law (Eq. (8)), an increase in the value of metal dissolution due to oxidation of anodes follows the increase in time, which consequently results in more energy consumption according to Eq. (6). Hence, a linear increase in operating costs with reaction time is observed. Since, the removal of *E. coli* and *C. perfringens* achieved 98.6% and 97%, respectively in 20 min, the *E. coli* and *C. perfringens* abatement did not match to WHO's potable water guidelines. Therefore according to Fig. 3d, for the abatement of 100% bacteria, 25 min of reaction time was considered in optimum condition.

3.2.6. Effect of initial bacteria count

To observe the effect of initial bacteria count on the bacteria abatement efficiency by EC, tests were performed for three different bacteria counts (10^3, 10^4, and 10^5 CFU/mL) for 25 min with constant CD of 33.3 A/m^2. As can be seen in Fig. 4a, with increasing count from 10^3 to 10^4 CFU/mL, the removal efficiency was constant (100%). However, the removal efficiency strictly decreased as the count increased from 10^4 to 10^5 CFU/mL and these were about 75% and 71% for *E. coli* and *C. perfringens*, respectively.

It should be noted that at constant CD and time, the value of produced $Al(OH)_3$ in all the solutions with different bacterial counts would be the same, and consequently, the same value of flocs would be generated in the solutions. Therefore, the flocs generated at high bacteria counts were insufficient for adsorption or trapping all of the bacterial cells. For the low count of bacteria, the number of $Al(OH)_3$ was higher compared to the number of bacterial cells. Therefore 100% bacteria removal was obtained in compared to higher count. Hence, it is quite clear that under the present operating conditions, the lower is the bacteria count better would be the abatement efficiency. Abadias et al. [26] reported similar results for the abatement of bacteria by EC process.

In this study, a one-way ANOVA analysis was applied to assess the statistical significance between the initial count of bacterial and residual count. As shown in Fig. 4b, there was a significant association between initial bacterial counts and abatement efficiency ($P < 0.01$; Table 3).

3.2.7. Energy and electrode consumption

The electrode consumed mass (g) in optimum conditions for each liter of potable water is achieved from mass difference of electrodes before and after EC. As expected, anode electrode had more mass loss as compared to cathode electrode. Therefore, the average consumed electrodes in treatment of potable water for Al and ST were 0.024 and 0.00174 g/L, respectively. As a result, the weight variation for cathode electrode is approximately negligible, which can be considered as zero. So the main cost for electrodes is associated with Al that must be considered in designing the water treatment systems. EEC (kWh/m^3 water) is an important economic parameter in EC process. EEC in EC process was calculated using Eq. (7). Table 4 presents EEC in different conditions, including CD (8.3–33.3 A/m^2), pH (7–9), reaction time (5–25 min), and initial bacteria count (10^4 CFU/mL). Table 4 describes the influence of CD on EEC. To increase CD in optimum conditions from 8.3 to 33.3 A/m^2 increased EEC from 0.2 to 2.259 kWh/m^3. The effects of initial pH on EEC in EC process is demonstrated in Table 4.

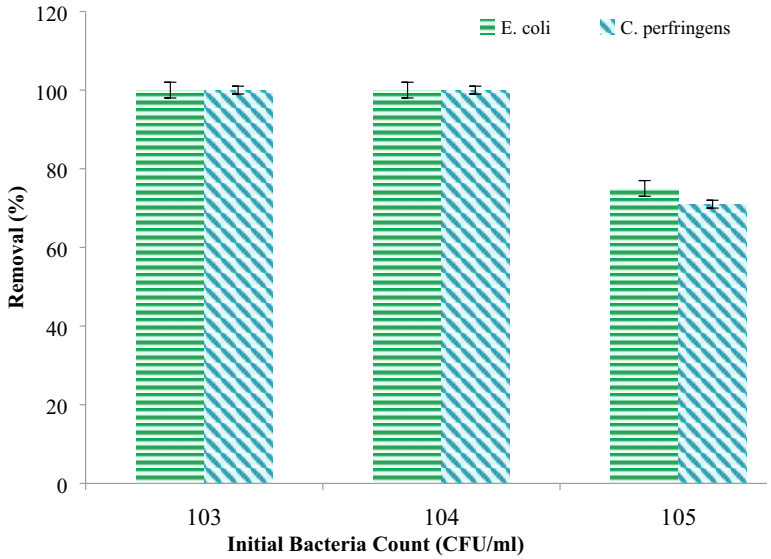

Fig. 4. Effect of initial bacteria count on removal efficiency of *E. coli* and *C. perfringens* (initial bacteria count: 10^3–10^5 CFU/mL, initial pH: 7, reaction time: 25 min, CD: 33.3 A/m^2, IED: 2 cm, and conductivity: 0.75 mS/cm). The mean value obtained from two separate experiments are demonstrated in data shown in the figure. Three replicates were carried out for every condition and the error bar is representative of standard deviation.

Table 3
One-way ANOVA analysis between initial counts of bacterial strains of *E. coli* and *C. perfringens* and their residual counts

	Source	DF	Adj. SS	Adj. MS	*F*-Value	*P*-Value
E. coli	Initial count	2	1,229,307,118	614,653,559	652.42	0.000
	Error	6	5,652,659	942,110		
	Total	8	1,234,959,777			
C. perfringens	Initial count	2	1,651,731,800	825,865,900	823.09	0.000
	Error	6	6,020,200	1,003,367		
	Total	8	1,657,752,000			

Table 4
EEC in different CD, reaction time, and pH (Initial bacteria count: 10^4 CFU/mL, conductivity: 0.75 mS/cm, and IED: 2 cm)

Current density (A/m^2)	Reaction time (min)	EEC (kWh/m^3)		
		7 = pH	8 = pH	9 = pH
8.3	5	0.041	0.0461	0.0417
	10	0.088	0.0931	0.0842
	15	0.1301	0.1403	0.1261
	20	0.171	0.1821	0.1628
	25	0.211	0.221	0.189
16.7	5	0.1302	0.1402	0.1406
	10	0.2601	0.2921	0.2931
	15	0.3901	0.4312	0.4344
	20	0.5213	0.5603	0.5645
	25	0.7	0.73	0.75
33.3	5	0.512	0.523	0.5334
	10	1.1124	1.1431	1.1998
	15	1.4211	1.5121	1.5236
	20	1.9351	1.9462	2.1635
	25	2.3156	2.4396	2.6381

It is obvious that EEC was not affected through primary pH, as the difference in EEC among the three tested pH was insignificant. As a conclusion, it could be stated that by considering removal efficiency (100%) for *E. coli* and *C. perfringens*, the optimum conditions for EEC are CD = 33.3 A/m^2 and reaction time = 25 min. As the obtained results for EEC investigation shows, the EEC increased with increasing CD and reaction time, which these results are in good agreement with those found in the study of Fayad et al. [14] and Kobya et al. [29]. In regard to optimum conditions selected, the EES was equal to 2.259 Kwh/m^3.

3.3. Morphological changes in E. coli cells and possible inactivation mechanisms

The antibacterial performance of EC process by assisted alum NCs is compared with that reported by other studies using other antibacterials. According to Table 5, the antibacterial performance of alum NCs is very effective compared with other antibacterials. To fully understand the mechanism responsible for the killing of *E. coli* by EC process, FE-SEM analysis was carried out regarding the samples of bacterial before and after treatment with EC process. The FE-SEM images of *E. coli* incubated in optimum conditions in presence and absence of the EC process were

obtained (Fig. 5). From the results of bacteria removal efficiency, we can conclude that binding of alum NCs to the cell surface was highly efficient. FE-SEM images (Fig. 5) show that *E. coli* bacteria are successfully trapped by alum NCs. The surfaces of majority of bacterial cells including *E. coli* are negatively charged in solution at pH 5.0–8.0 [46,47]. Hence the bacteria can be adsorbed quickly by electrostatic force on alum NCs surface [48]. Fig. 5a shows that before the EC process, there are a large number of bacteria in solution. The number of bacteria becomes significantly lower after 15 min reaction time (Fig. 5b). It is clear that a large amount of bacteria have been killed. After 25 min reaction time, almost no bacteria have survived. Figs. 5a and d show the structure of the control sample (*E. coli* cells incubated in NC-free). The bacterial cells were in sizes of normal with intact membrane structures. However, cells growing in the aqueous media underwent 25 min reaction time with EC process show a very rough surface and expanded structure (Fig. 5e). Also, cells (Fig. 5f) were

Table 5
Comparison of antibacterial performance of EC process by assisted alum NCs with different disinfectants

Disinfectant	Bacterial killing (%)	References
Polymer-filter paper	99	[48]
Electrocoagulation	97	[33]
Electrochemical	99	[49]
Electrocoagulation	99.73	[13]

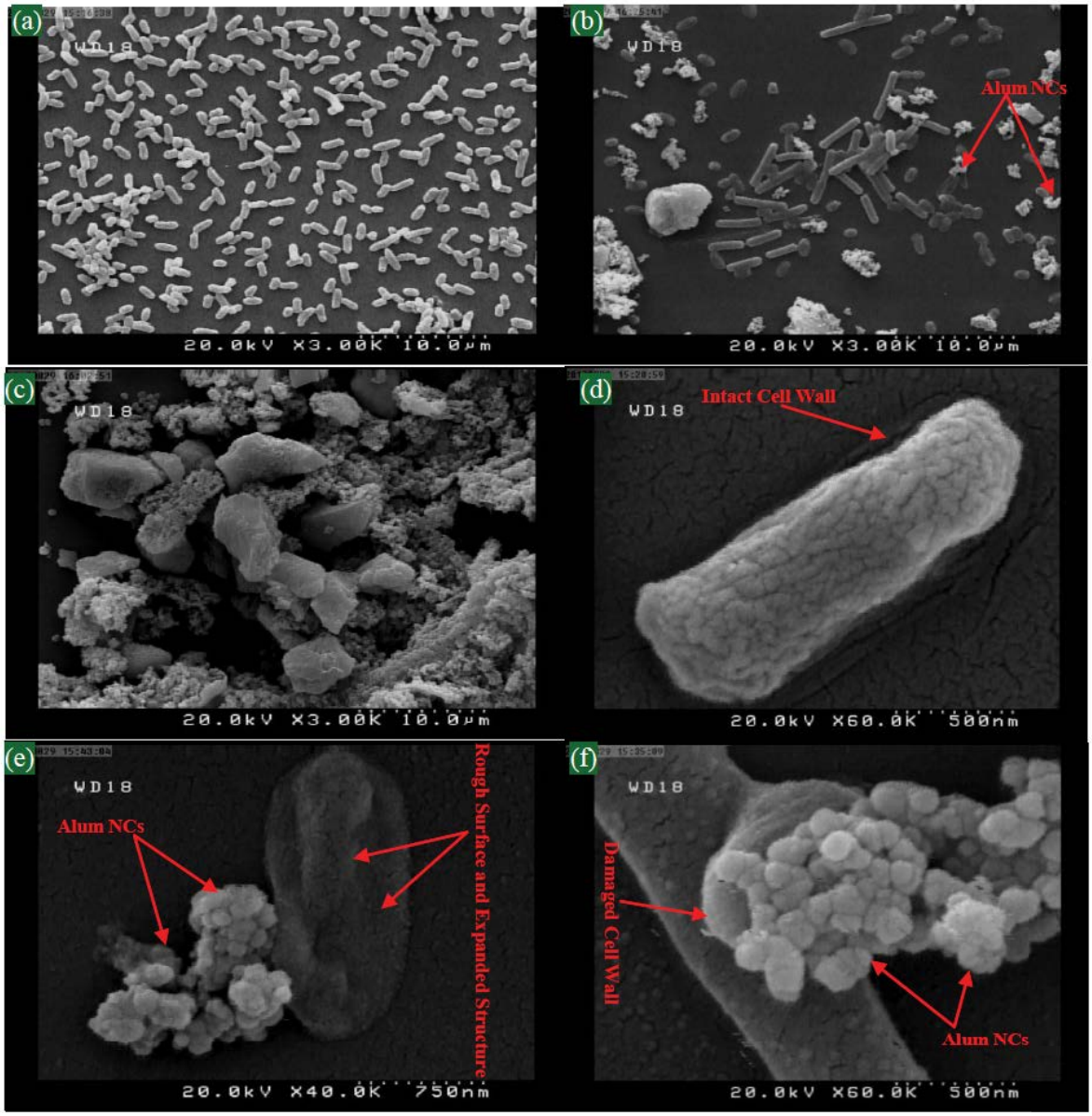

Fig. 5. FE-SEM images of (a and d) *E. coli* (control) and (b, c, e, and f) *E. coli* obtained after 15 and 25 min reaction time with alum NCs.

clearly damaged by alum NCs. The cell wall of the bacteria captured by the alum NCs is approximately damaged. Moreover, it is clear that alum NCs have penetrated the membrane and destroyed its structure. Based on the results of bacteria-alum NCs binding, it might be suggested that the smaller size alum NCs capture bacteria upon contacting the cell wall and finally kill them by destruction of the cell wall. Also, suggesting that intense damages have happened to the bacteria. Alum NCs produced from the anode surface attack the outside of the bacteria, also H_2O_2 and free radicals, such as $O_2^{\bullet-}$ and $^{\bullet}OH$, that could be generated during EC, can damage nucleic acids, proteins, etc. [36]. The bacterial membrane is composed of phospholipids' layers with hydrophilic and hydrophobic parts, which preserve the vital centers of the bacteria cells. To destruct the phospholipid membrane, an oxidant with the ability to pass via a membrane and reach vital centers is needed [49].

Also, bacteria can be removed by adsorption on electrodes. Many investigations showed that the lipopolysaccharide molecules on extracellular membrane of gram-negative bacteria can be adsorbed on anode electrode due to negative charge [36,50]. However, the mechanism underlying the destruction of bacteria by EC is not fully obvious yet.

3.4. Characterization of alum nanocrystallites and sludge

To characterize the specific surface area of alum NCs, BET technique, and nitrogen adsorption/desorption isotherms were applied. N_2 adsorption/desorption analysis for alum NCs are presented in Fig. 6a. The surface area is 109.79 m^2/g (BET method). Pore volume and pore diameter of alum NCs were 25.225 cm^3/g and 2–50 nm (mesoporous with a mean pore diameter of 13.698 nm), respectively. The study carried out by Wang et al. [31], about the utilization of alum sludge for producing aluminum hydroxide (chemical coagulation), indicated that as the temperature rises (70°C–170°C), the surface area and pore volume increase. Surface area and pore volume were increased from 5.8 to 95.6 m^2/g and 0.008 to 0.414 g/cm^3, respectively. In a study carried out on BET surface area and pore structure of dewatered alum sludge cakes (chemical coagulation), it was shown that surface area of fresh alum sludge was 49.03 m^2/g [51].

In EC the electrolytic oxidation of an appropriate anode material generates the active coagulant species, thus differing in chemical coagulation in which coagulants of chemical such as polyelectrolytes and metal salts are used [52]. These distinct methods in fact lead to various chemical environments, which should have effect on surface area and pore structure of alum NCs. High surface area and pore volume of alum NCs causes higher activity of alum NCs in aqueous solution and subsequently higher efficiency in bacteria abatement. Figs. 6b and c (FE-SEM) show two various magnifications of alum NCs.

Figs. 6b and c show that alum NCs have sizes about 80 nm with uniform morphology and shape which can act as good alum NC to remove bacteria. The figure obviously displays that the surface of alum NCs has many pores and also because of their multi-layered structures with high porosity, they can easily absorb the bacteria. The TEM images of the alum NCs are shown in Figs. 6d and e. Alum NCs were shown to have a spherical shape with a mean diameter size of 80 nm.

The crystalline structures of the alum NCs were identified with XRD (Fig. 6f). For alum NCs, diffraction peaks with 2θ at 18.45°, 20.46°, 27.79°,37.47°,40.54°, 44.79°, 52.53°, 58.41°, 63.95°, and 70.93° were observed, these points were related

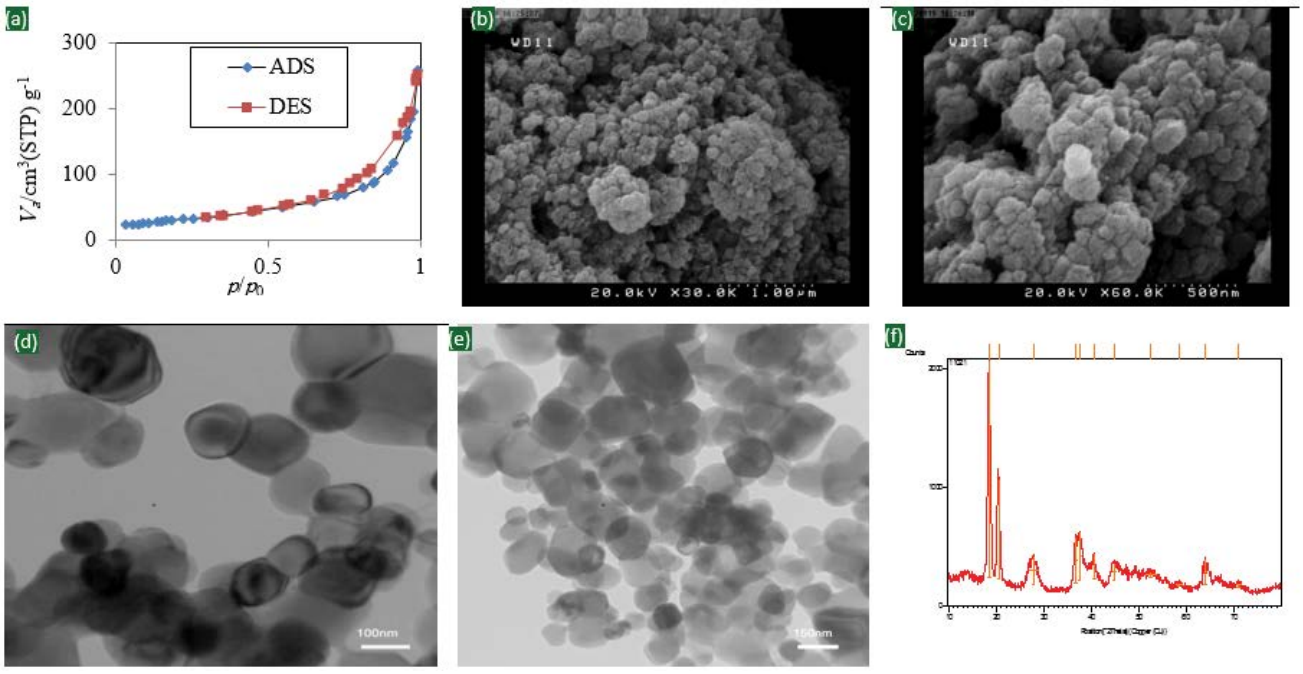

Fig. 6. BET plots of nitrogen adsorption–desorption isotherms (a), FE-SEM image at different magnifications (b and c), TEM Image at different magnifications (d and e), and X-ray diffraction patterns (f) of the Alum nanocrystallites.

Table 6
Constituents of electrocoagulation sludge

Constituents	%
Al_2O_3	62
Fe_2O_3	0.55
CaO	0.073
Na_2O	0.187
MgO	0.904
K_2O	N
TiO_2	0.032
MnO	0.05
P_2O_5	0.017
L.O.I	35

to NCs of boehmite AlOOH. The particle sizes of the NCs of boehmite AlOOH using the Scherrer equation [53] were 90 nm. The combination of NCs of AlOOH was detected and the existence of alum NCs-damaged bacteria (Figs. 5e and f) proves that their structure is related with a strong antibacterial activity.

Considerable bacteria adhesion to the surface of the electrogenerated alumina, which is promoted by the nanostructured structure of alumina, is considered to be the fundamental effect in the bacteria abatement of EC process. Thereafter, strong bacteria abatement is concomitant with the alumina sedimentation. The EC sludge compounds obtained using the XRF analysis are shown in Table 6. Loss on ignition (L.O.I) method was used to estimate organic matter and carbonate content of EC sludge after heating the sample. The value of loss on ignition was achieved to be 35%. This contribution of sludge can be arising from precipitated and adsorbed bacteria and also from by-product of destruction. A large contribution of sludge (62%) is attributed to Al_2O_3. Presence of phosphorus pentoxide (P_2O_5) may confirm the decomposition of bacteria during EC process.

4. Conclusion

Batch EC studies with several experimental factors such as reaction time, initial pH, CD, IED, and conductivity were carried out to survey the influence of EC on the abatement of *E. coli* and *C. perfringens* from potable water.

In accordance to the results, the EC time at 25 min was sufficient for the abatement of 100% of bacteria (both *E. coli* and *C. perfringens*) by considering other conditions of IED of 2 cm, initial pH of 7, CD of 33.3 A/m^2, conductivity electrical of 0.75 mS/cm and initial bacteria count of 10^4 CFU/mL with an operating cost of 0.6 USD/m^3 treated water. Increasing CD can significantly remove *E. coli* and *C. perfringens* strains in potable water resources. Bacteria removal efficiency was decreased when the initial bacteria count were more than 10^4 CFU/mL. IED has a negligible impact on abatement efficiency of bacterial strains. In the EC process, the temperature rises as time proceeds, which can be controlled with the selection of a suitable conductivity. Alum NCs play an essential role in influencing the antibacterial efficiency, which is presumably due to the electrostatic forces. EC process is economically viable for the removal of *E. coli* and *C. perfringens* from potable water in comparison to the existing industrial disinfection processes. Cell wall damage and expansion were found on the *E. coli* bacteria after 25 min EC process. These results demonstrate that EC process could be potentially considered as an effective antibacterial agent for protecting potable water safety.

Acknowledgments

This study is related to the project NO. 95-03-193-29467 from Student Research Committee, Iran University of Medical Sciences, Tehran, Iran. We also appreciate the "Student Research Committee" and "Technology Talent" in Iran University of Medical Sciences for their financial support of this study.

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