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Escherichia coli inactivation using a hybrid ultrasonic–electrocoagulation reactor

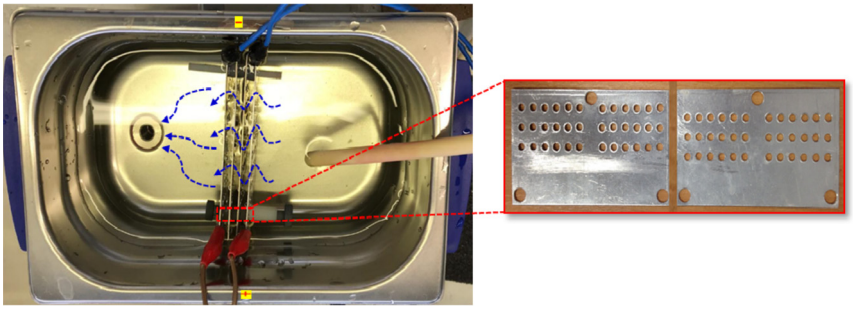
Khalid S. Hashim [a, b, *], Shaimaa Satae M. Ali [b], Jawad K. AlRifaie [c], Patryk Kot [a], Andy Shaw [a], Rafid Al Khaddar [a], Ibijoke Idowu [a], Michaela Gkantou [a]

[a] BEST Research Institute, Liverpool John Moores University, Liverpool, UK
[b] Department of Environment Engineering, Babylon University, Babylon, Iraq
[c] Al-Furat Al-Awsat Technical University, Kufa, Iraq

HIGHLIGHTS

- The new hybrid U-E reactor removed 100% of *E. coli* within 11 min at low cost.
- U-E reactor reduces the power consumption by about 56%.
- Application of ultrasonic field prevents the growth of anodic passive layer.
- The new design of electrodes eliminates the need for external water mixers.

GRAPHICAL ABSTRACT



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ABSTRACT

In the current study, a new hybrid ultrasonic-electrocoagulation reactor (U-E reactor) has been used to inactivate *Escherichia coli* in water. The new hybrid reactor consists of an ultrasonic bath fitted with four perforated aluminium electrodes. These perforated electrodes are designed to act as baffle-plates to enhance the water-mixing process. The electrodes eliminate the need for external mixing devices, which in turn, enhances the cost-effectiveness of the unit. Initially, the ability of the electrocoagulation to inactivate *E. coli* was optimised for different operating parameters such as electrolysing time (T_e), electrodes spacing (ES) and current density (CD). The ultrasonic field was then applied over different time periods (T_u), during the course of the electrolysing process. Statistical analyses have been conducted to assess the relative effect of each operating parameter on the inactivation of *E. coli*. An economic study has also been conducted to assess the operating costs of the U-E reactor. The results revealed that the new U-E reactor inactivated 100% of the *E. coli* within 11 min of electrolysis at ES of 5 mm, CD of 1.5 mA/cm^2, and an operation cost of 0.212 US $/m^3. It was been established that the relative effect of operating parameters on *E.coli* inactivation followed the order: $T_e > T_u > CD > ES$.

1. Introduction

Planet Earth faces intolerable water scarcity for several reasons such as the rapid growth in both world population and industries both which consume huge quantities of fresh water, at the same

* Corresponding author. BEST Research Institute, Liverpool John Moores University, Liverpool, UK.
E-mail address: k.s.hashim@ljmu.ac.uk (K.S. Hashim).

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time, producing significant amounts of polluted water (Bizzi et al., 2019). The challenge of addressing water scarcity is heightened by increases in the cost of developing new water sources, water pollution and the depletion of sources of fresh water (Hanjra and Qureshi, 2010; Jung et al., 2015). As a result, water pollution-related risks threaten public health on a the global scale (Hanjra and Qureshi, 2010; Jung et al., 2015). To reverse this increasing trend in water pollution, a wide range of water treatments, monitoring and sensing methods have been developed and practised over the last few decades (Agi et al., 2019; Ryecroft et al., 2019; Wang et al., 2019).

Regarding these forms of water pollution, pathogenic and non-pathogenic microorganisms are categorised as high-risk pollution as they cause a variety of serious waterborne diseases, such as diarrhoea and gastrointestinal disorders, which are responsible for millions deaths per year (Anese et al., 2015; Ramirez-Castillo et al., 2015; Baran et al., 2018). As such, different disinfection methods, such as chlorination, ozonation, electrocoagulation, ultrasonic, and ultraviolet application have been practiced to remove biological pollutants from water and wastewater (Alattabi et al., 2017; Ohrdes et al., 2018; Hashim et al., 2020). Chemical disinfection methods were applied extensively during the 1970s as an effective and cost-effective solution for biological pollution (Castro-Rios et al., 2014), these methods mainly depending on the powerful oxidizing ability of chemicals such as chlorine, to destroy microorganism enzymes (Castro-Rios et al., 2014). However, it has been found that these methods produce toxic by-products such as trihalomethanes (Bidhendi et al., 2006; Castro-Rios et al., 2014). Advanced filtration techniques, such as ultrafiltration, were also applied over the last few decades to remove microorganisms from water. The application of this technique however, is limited due to high operational costs and technical problems such as the fouling (Bagga et al., 2008).

Ozone is categorised as a powerful oxidant that can be used as an effective water disinfection method (Bidhendi et al., 2006). Crucially, ozonation process does not generate trihalomethanes by-products but both the high operational costs of this method and the low solubility and stability of ozone in water, limit the application of this method for water treatment (Wang and Bai, 2017). Recently, many advanced techniques have been developed to disinfect water, such ultraviolet irradiation, electrocoagulation and ClO_2. However, the application of these methods on a large scale, is limited by several factors, such as operational costs and compulsory pre-treatment procedures (Ghernaout et al., 2008; Hashim et al., 2018). In reality, the relevant literature indicates that there is no single treatment method which can achieve a complete, efficient and cost-effective disinfection process.

The current study therefore, investigates the application of a new hybrid ultrasonic-electrocoagulation reactor (U-E reactor) to inactivate *E. coli* in water. *E. coli* is the main species in the faecal coliform group, and as such is usually used as an indicator micro-organism to evaluate water quality (Li et al., 2017a, 2017b). The electrocoagulation method (EC) has been chosen due to its simplicity, high efficiency and relatively low operating cost (Nidheesh and Singh, 2017; Danial et al., 2019). This method does not required chemical additives and could easily be automated and integrated with other treatment methods (Kumar et al., 2018; Rosales et al., 2018). It also minimises the volume of solid waste (sludge) generated which enhances its cost-effectiveness (Baran et al., 2018).

2. Materials and methods

2.1. Experimental set up

The experimental work has been carried out using a 2.75 L ultrasonic bath (Fisherbrand, model: FB15051), supplied with four perforated electrodes (Fig. 1). The perforated electrodes are made from aluminium of 99.5% purity, which are 10 cm wide, 6.5 cm high and 0.1 cm thick. Each electrode contains 36 holes, 0.4 cm in diameter, distributed in twelve-hole lines. The holes in the cathode were offset from the holes in the anode by 0.5 cm, to allow the water being treated to flow in a convoluted path, this helping to mix the water. The electrodes were insulated from the interior surfaces of the ultrasonic bath using PVC rods (10.5 mm in diameter). This design of electrodes eliminates the need for external water mixing apparatuses, which enhances the cost-effectiveness of the unit. The total effective area of the electrodes immersed in water, was 207 cm^2.

A peristaltic pump (type: Watson-Marlow, model: 504U) was used to pump water, the required current density supplied by a DC power source (type: HQ rectifier, Model: PS 3010). A portable pH-temperature device (Type: Hanna; Model: HI 98130) was used to measure both the temperature and pH of the water.

2.2. Preparation of E. coli contaminated water sample

Synthetic water samples were prepared according to (Hooshyari, 2017). Initially, the *E. coli* (ATCC 35218 supplied by Fisher Scientific) was cultured in a flask containing a 0.1 L Luria Broth Base which was prepared by dissolving 10 g of tryptone, 5 g of yeast extract, and 5 g of sodium chloride in 1000 ml of deionised

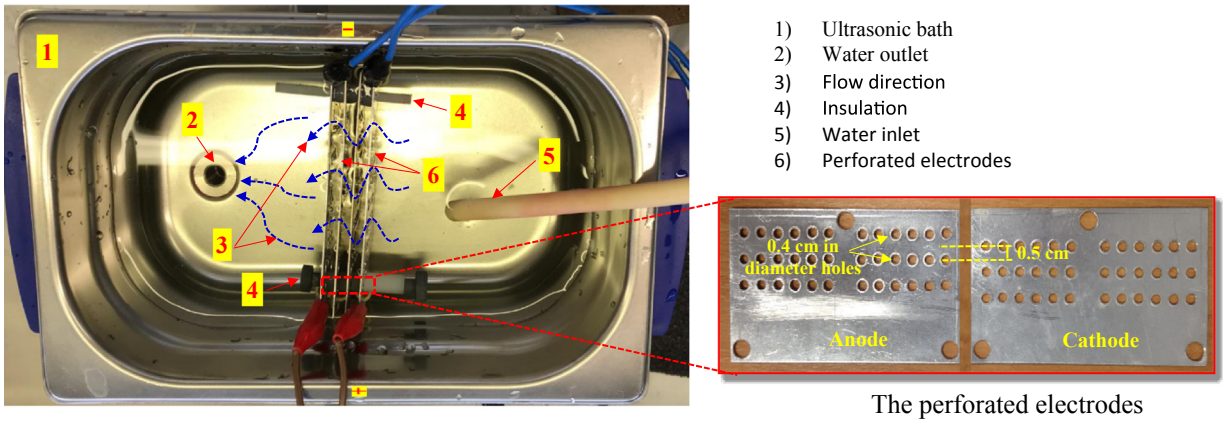

Fig. 1. The U-E reactor.

water. The incubation process was carried out by shaking the flask at 150 rpm for 24 h at a temperature of 37 °C using a temperature-controlled shaker. A centrifugal process was applied to separate the growth media from the cells. The separated cells were washed with a buffer solution and then suspended in said buffer solution. The latter was prepared using 0.01 and 1.0 mol of KCl and $NaHCO_3$ respectively, in 1000 mL of distilled water. The initial concentration of the *E. coli* was 10^8 UFC/100 mL. Lower concentrations (10^5 UFC/L) were diluted from this stock solution. After each dilution process, the caps and top edges of the bottles were sterilised by flame to avoid pollution with external types of bacteria.

2.3. Experimental work

The mixing process is an essential process in water treatment because it enhances the chance of contact between coagulants and pollutants, this resulting in quicker growth of flocs (Yu et al., 2011). In the current study, perforated baffle-plates were used to enhance the water mixing process thus negating the need for external mixing devices. The mixing efficiency of the new EC electrodes was monitored in continuous flow mode and compared to the mixing efficiency of traditional electrodes. Traditional EC electrodes are similar in design to the new EC electrodes, but without the holes.

Water mixing efficiency has been assessed by placing both the new and traditional electrodes in transparent containers (made from Perspex with net width of 10 cm), filled with clear deionised water. Coloured water (400 mg/L of red drain dye) was then pumped continuously through these containers at a constant flow rate of 30 mL/min for 30 min. The flow of the coloured water through the new and traditional reactors was continuously monitored using HUE HD cameras which were installed at a distance of 30 cm from the reactors. The camera records then were separated into frames using VirtualDub software. The unmixed areas on these frames, were measured using AutoCAD-2014 software.

The inactivation of *E. coli* was initiated by measuring the initial pH of the diluted samples, this around 7. The initial pH of the treated samples was kept at 7 because the initial pH of both surface water and municipal wastewater ranges between 6 and 8 (Cohen and Kirchmann, 2004; WEF, 2007) and all experiments were run at room temperature (20 ± 1 °C). The experimental work was divided into two phases, the first phase focusing on the ability of the electrocoagulation to inactivate *E. coli,* taking into account the influence of electrolysing time (T_e), electrodes spacing (ES) and current density (CD). This phase was carried out by treating 1 L of the prepared sample over different electrolysing times (from 5 to 30 min), a range of spaces between electrodes (5–15 mm) and varying current densities (0.5–2.5 mA/cm^2). The progress of *E. coli* inactivation was monitored by collecting 2 mL of water treated at different times (0, 5, 10, 15, 20, 25, and 30 min) during the course of the experiment, to calculate the number of surviving *E. coli* cells. The following equation was used calculate the *E. coli* inactivation efficiency (RE %):

$$RE\% = \frac{C_i - C_f}{C_i} \times 100\% \quad (1)$$

where C_i and C_f are the influent and effluent number of *E.coli*, respectively.

The second phase of this study concerns the application of an ultrasonic field to enhance the inactivation of the *E.coli*. In this phase, the ultrasonic field (0.28 kW and 37 kHz), was applied simultaneously with the electrolysing process for two different periods of time: 5 and 10 min. The power and frequency of the ultrasonic field (0.28 kW and 37 kHz) were chosen because these values have previously been used for the degradation of different pollutants in water and wastewater (Neppolian et al., 2002; He et al., 2007; Song et al., 2007; Shriwas and Gogate, 2011; Reddy et al., 2016).

The progress of *E. coli* inactivation was monitored using the same procedures as described above. The results from these two phases were compared to check the effects of the ultrasonic field on the inactivation of *E.coli*.

2.4. Operating costs

In lab-scale work, estimations of operating costs usually include the costs of power consumed, materials and chemicals. In the current work, the operating cost of the new U-E reactor in terms of *E. coli* inactivation, was estimated according to the method used by Hashim et al. (2017a), which is summarised by the following equation:

$$Operating\ cost = \gamma_{electrodes} \times Q_{electrode} + \gamma_{power} \times Q_{power} \quad (2)$$

where, $\gamma_{electrodes}$ represents the price of the electrode material, and $Q_{electrode}$ (kg of Al/m^3) the consumed weight of said material. The power consumed can be calculated as follows:

$$E = \frac{I*V*t}{Vol.} \quad (3)$$

where *E, I, V, t and vol.* represent the consumption of electrical energy (kWh/m^3), current (A), potential (V), electrolysis time (hrs) and volume of solution (m^3), respectively.

2.5. Relative effects of the operating parameters

The relative influence of each parameter (T_e, ES, CD, and T_u) on the inactivation of *E.coli* from water using the new U-E reactor, has been statistically analysed. Beta coefficient (β) was used to measure the relative influence where the higher the β, the higher the influence (Hashim et al., 2017b). The Beta coefficient shows whether the studied parameter has a significant influence on the inactivation of *E.coli* or not. An SPSS-23 package has been used to analyse the experimental results and to determine β values.

3. Results and discussion

3.1. Phase I: Inactivation of E.coli using the electrocoagulation method

3.1.1. Mixing efficiency of the new EC electrodes

The results obtained from the analyses of the camera records show that the new EC electrodes required 15 min to create a homogenous colour distribution across the whole reactor (Fig. 2). 30 min was not enough for the traditional EC electrodes to create a homogenous colour distribution. These results indicate the cost-effectiveness of the new EC electrodes as they achieved efficient water mixing without using external mixers.

3.1.2. The influence of treatment time

Several tests were used to investigate variations in *E.coli* inactivation according to electrolysing time which was fixed at 30 min 2 mL samples were collected at 5, 10, 15 and 20 min, the CD, SE and initial pH kept at 0.5 mA/cm^2, 0.5 cm and 7, respectively. During the first 5 min of treatment, white froth developed on the surface of water. Between 7 and 10 min, the colour of the solution changed to light yellow and the thickness of the froth increased. For the remainder of treatment time, the froth became white, while the solution became clear and transparent. These results (Fig. 3),

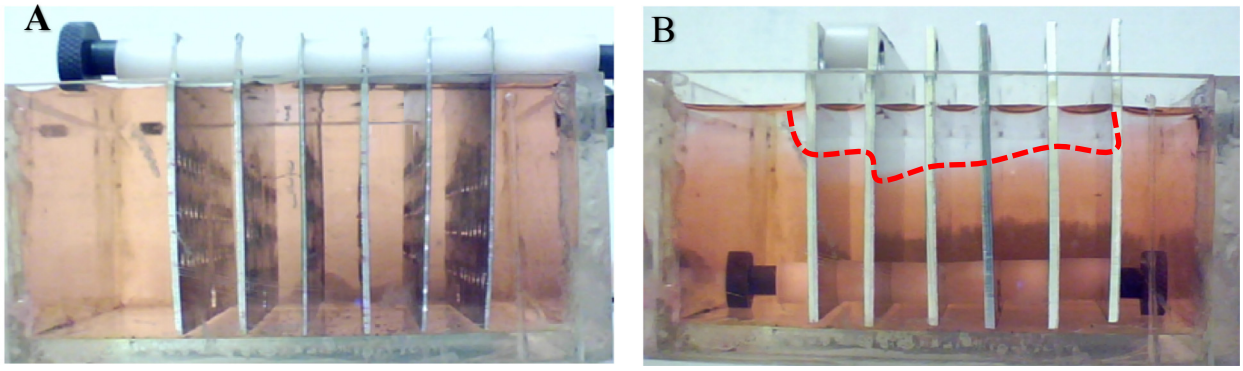

Fig. 2. Mixing efficiency of (A) new EC electrodes, (B) A traditional EC electrodes.

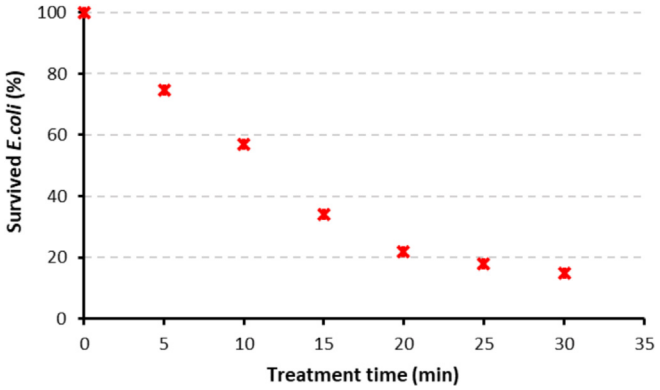

Fig. 3. Influence of Te on *E.coli* inactivation.

indicate that the number of residual (surviving) *E.coli* cells decreased rapidly from 100% to approximately 21% during the first 20 min of treatment, slowly decreasing over the remaining treatment time. This increase in the deactivation of *E.coli* cells during the first 20 min of treatment, could be attributed to two effects: direct and indirect. The first effect is the direct effect of the electric current that causes potential changes on the cellular membrane, consequently destroying the membrane (Li, 2004). Indirect effects are caused by contact between the *E. coli* and the produced oxidants (Drees et al., 2003), and/or the adhesion of the *E. coli* to flocs that result in removal from the solution being treated, either by floatation or sedimentation (Ricordel et al., 2014). The decrease in the removal efficiency during the last 10 min of treatment can be attributed to the development of a passive layer on the surface of the aluminium anodes that decreases anode dissolution, this in turn decreasing the removal of the targeted pollutant (Lu et al., 2015). As such, a treatment time of 20 min will be used to commence the experimental work.

3.1.3. *Influence current density (CD)*

To understand the effect of CD on the performance of the electrocoagulation method, the inactivation of *E.coli* was investigated at three different CDs: 0.5, 1.5, and 2.5 mA/cm^2, keeping the initial pH, ES and T_e constant at 7, 0.5 cm, and 20 min, respectively. The results confirmed that the inactivation of *E.coli* is enhanced by an increase in CD. It can be seen from Fig. 4(A) that when the CD increased from 0.5 to 2.5 mA/cm^2, the ratio of surviving *E.coli* decreased from about 20% to 0% within 20 min of electrolysing. As was stated above, an increase in inactivation of *E.coli* due to an increase in current density, can be attributed to several factors. Firstly, increasing the CD increases potential difference on the cellular membrane and restricts the movement of ions through the cell wall, this destroying essential physiological functions in the cell (Ghernaout et al., 2008). Secondly, proteins in the phospholipidic membrane of the living cell can be easily oxidised by the electrical current, this leading to the inactivation of the living cells (Drees et al., 2003). Finally, the number of coagulant ions generated increases with an increase in CD, this also enhancing removal efficiency. However, Fig. 4(B) shows that increasing the CD, increases the consumption of power. Because of this, a CD of 1.5 mA/cm^2 will be used as the optimum value in the current investigation.

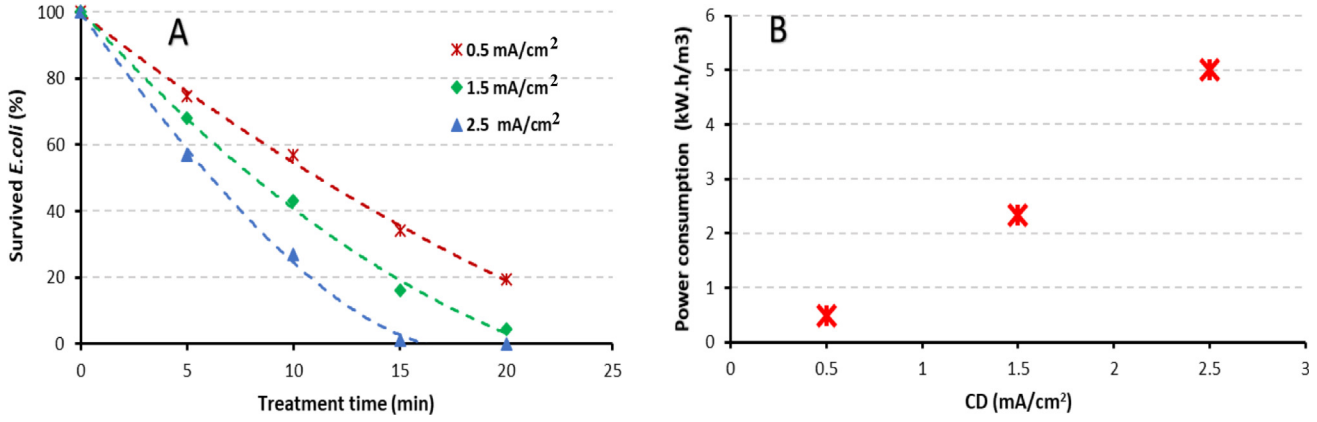

Fig. 4. Effect of CD on: (A) *E.coli* inactivation, (B) Power consumption.

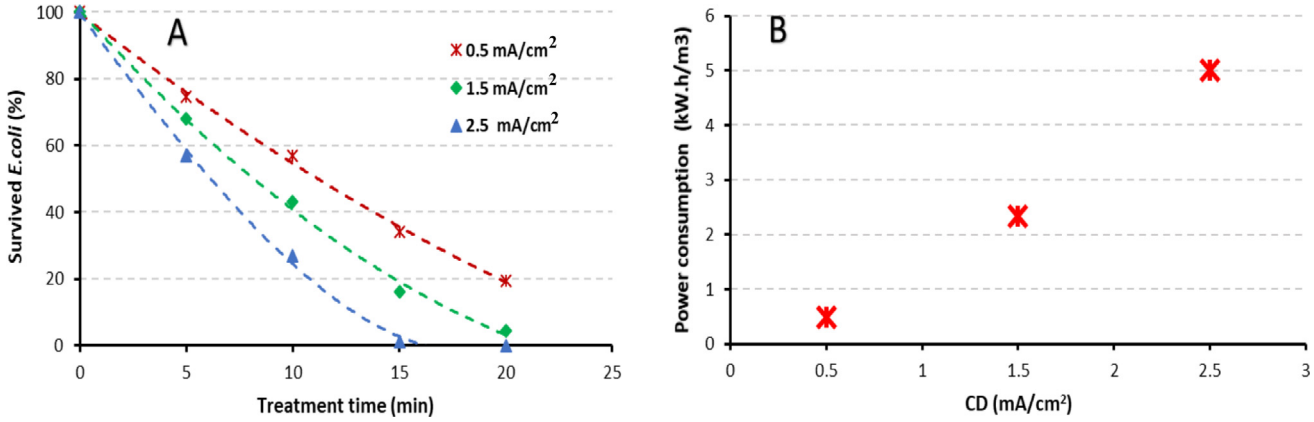

Fig. 5. Effect of ES on: (A) *E.coli* inactivation, (B) Power consumption.

3.1.4. The influence of electrode spacing (ES)

The space between electrodes, plays a vital role in the removal of the targeted pollutants as it determines both the electrical resistance and the development of the passive layer on the surface of the anode. As such, three different spaces between electrodes (0.5, 1, and 1.5 cm) were studied. The initial pH, CD and T_e were kept constant at 7, 1.5 mA/cm^2, and 20 min, respectively. The results revealed an inverse proportional pattern between the inactivation of *E.coli* and ES. The ratio of the surviving *E.coli* increased from approximately 4%–28% as the ES increased from 0.5 to 1.5 cm, Fig. 5(A). This reduction the inactivation of *E. coli* could be explained as the increase in the space between electrodes, enhancing the growth of a passive layer on the surface of anodes. This increases the electrical resistance, which in turn minimises the inactivation of *E. coli*. Fig. 5(B) suggests that increasing ES does not benefit the electrocoagulation method as it maximises power consumption. Therefore, 5 mm ES was used as the optimum value for *E.coli* inactivation.

In comparison to the relevant literature, the general findings of this phase of the current study show good agreement with previous studies. For example, similar results trends were found by Ghernaout et al. (2008).

In conclusion, the outcome of the current phase of study indicate that the electrocoagulation method can inactivate approximately 96% of *E. coli* from water within 20 min of treatment at an initial pH of 7, CD of 1.5mA/cm^2 and ES of 0.5 cm.

It should be noted that the final pH of the solution being treated, has increased from 7 to about 10 after 15 min of electrolysing. This increase in pH indicates that sweep coagulation is the predominate path because in a high alkalinity environment, the coagulant agents do not have high positive charges, meaning the adsorption or charge neutralization paths will not be very effective (Zhao et al., 2011).

3.2. Phase II: Inactivation of E.coli using U-E reactor

This phase was carried out to highlight the novelty of the current approach: the ultrasonic field will be simultaneously applied alongside the electrolysing process to enhance the inactivation of *E. coli*. In this phase, the ultrasonic field (0.28 kW and 37 kHz), will be applied at two different durations (T_u), 5 and 10 min, at the beginning of electrocoagulation process. The electrolysing process will be carried out using a CD of 1.5 mA/cm^2, ES of 0.5 cm and initial pH of 7, these being the optimum values identified in the previous phase.

The results from this phase of study confirmed that the ultrasonic field significantly enhanced the inactivation of *E. coli* from water. Fig. 6 shows that application of ultrasonic field for 5 and 10 min shortened the required treatment time from 25 min to 15 min and 11 min, respectively. This means that application of the ultrasonic field for 10 min, shortens the treatment time by approximately 56%. This enhancement in *E.coli* inactivation can be attributed to several factors. The first is due to the development of high pressure and temperatures inside the solution being irradiated. It has been reported that ultrasonic irradiation produces a high number of microscopic bubbles that collapsed inside the solution, causing a sudden increase in the pressure and temperature in their vicinity (Doosti et al., 2012). The elevated pressure and temperature damages the cell wall and disrupts the cell membrane this resulting in the death of microorganisms (Liu et al., 2011). Another mechanism is that of the diffusion of chemicals into the cell due to the damage of the cell wall. Related studies indicated that ultrasonic irradiation can cause cuts or damages to the cell walls, this allowing harmful chemicals to diffuse into the cell, resulting in the death of microorganisms (Joyce et al., 2003). The ultrasonic field efficiently cleans metallic surfaces (Long et al., 2019), meaning that its' application prevents the growth of a passive layer on the surface of anodes, which in turn enhances anode dissolution, consequently enhancing removal efficiency.

Scanning electron microscopy (SEM), coupled with energy-dispersive X-ray (EDX), have been used to analyse the composition of the flocs produced. These analyses indicated that aluminium, carbon, nitrogen and oxygen represent 15.3%, 40.2%,

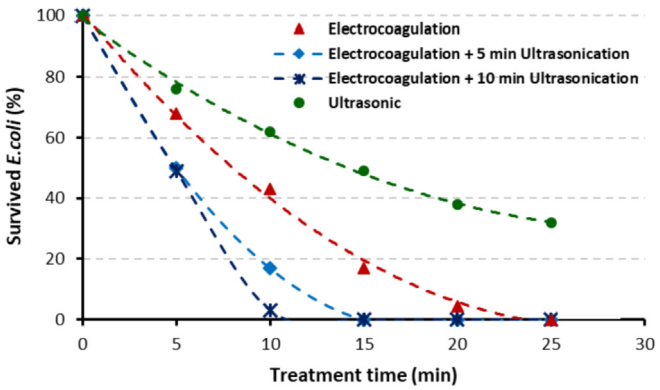

Fig. 6. Influence of ultrasonic field on *E.coli* inactivation.

8.5% and 20.1% of the chemical composition of the flocs produced, respectively.

3.3. Operating cost

The actual operating cost of any treatment method must include the costs of the power consumed, chemicals and electrode materials (Ghosh et al., 2008). It must cover the cost of labour, treatment of sludge, maintenance and fixed costs such as the basin and pipes (Ghosh et al., 2008). However, for a lab-scale unit, the operating cost is usually a preliminary estimate that should cover the cost of power, electrode materials and chemicals (Kobya et al., 2010), an estimate of these made for the current study. . The estimation has been carried out according to the unit prices in the Iraqi market in June 2019 where power costs 2.5 cent/kWh and cost of 1 kg of aluminium is 1.53$). According to equations (2) and (3), the preliminary operating costs of *E.coli* inactivation using the new U-E reactor is 0.212 $/m^3.

The operating cost of the new U-E method is comparable to the operating costs of traditional methods. It has been reported that the operating cost of electrocoagulation method is about 0.2 $/m^3 (Hashim et al., 2017c), which slightly cheaper than the cost of the new U-E method. This because the new U-E reactor shortens the treatment time by 56% in comparison with traditional electrocoagulation reactors.

3.4. Relative effects of operating parameters

The relative influence of each one of the studied parameters on the inactivation of *E.coli* from water, using the new U-E reactor, has been measured using a β coefficient. The calculated values of the β coefficient indicated that the electrolysing time exerts the highest influence (42%), followed by Ultrasonication time (30%) and applied current density (20%) (Fig. 7). The space between electrodes exerts the lowest influence (8%).

It is suggested that this new method can be used for the treatment of effluents from biological laboratories, hospitals and private clinics. Because this new method can be applied using renewable energy sources such as solar panels, it can be used in emergencies and for small communities in poor countries where surface or ground water which has nt been properly treated, is consumed.

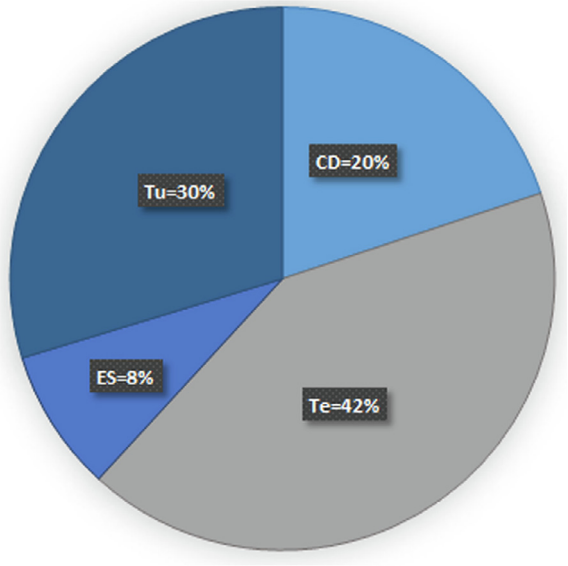

Fig. 7. Relative influence of the studied parameters on the inactivation of *E.coli*.

4. Conclusion

The ability of a new hybrid ultrasonic-electrocoagulation reactor which can inactivated pathogens (*E.coli*) present in water, has been investigated. The results confirm that the new U-E reactor could be a useful and cost-effective alternative to traditional, water-disinfection methods. It has been found that the survivability of *E. coli* significantly decreases with an increase of electrolysing time, Ultrasonication time or applied current density. In contrast, increasing the space between electrodes negatively influenced the inactivation of *E.coli*. The results also indicated that the most effective operating parameters for the inactivation of *E. coli* by the new method, are electrolysation and Ultrasonication time, while the space between electrodes has the lowest impact on the removal process. Finally, for future work, it is necessary to investigate the mechanisms of *E. coli* inactivation using the new U-E reactor.

CRediT authorship contribution statement

Khalid S. Hashim: Conceptualization, Methodology. **Shaimaa Satae M. Ali:** Formal analysis. **Jawad K. AlRifaie:** Software, Methodology. **Patryk Kot:** Data curation. **Andy Shaw:** Project administration. **Rafid Al Khaddar:** Supervision. **Ibijoke Idowu:** Writing - original draft. **Michaela Gkantou:** Validation, Writing - review & editing.

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