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Electrosorptive disinfection of *Escherichia coli* (*E. coli*) aqueous solutions by activated carbon monolith electrodes

Edip Bayram, Ceren Karaman, Zehra Kuru and Onur Karaman

ABSTRACT

Electrosorption, which can be defined as adsorption onto the surfaces of charged electrodes, has been developing as an efficient and environmentally friendly technology for removing toxic pollutants from aqueous solutions. In this study, an industrial process was used for the fabrication of activated carbon electrodes (ACEs). An electroless metal deposition method was used for the modification of activated carbon granules with silver (Ag) for antibacterial activity of electrodes. The antibacterial activity of Ag-modified–ACEs (Ag–ACEs) for *Escherichia coli* (*E. coli*) bacteria commonly found in water was tested. Adsorption and electrosorption behaviors of *E. coli* aqueous solutions onto ACEs and Ag–ACEs were examined in a cyclic electrosorption system. It has been concluded that the performance of Ag–ACEs is better than ACEs as an electrode for electrosorption of *E. coli*. Moreover polarization can significantly enhance the removal efficiency of *E. coli* on both ACEs and Ag–ACEs. Finally, electrosorption capacity of the system for *E. coli* was determined.

Key words | activated carbon electrode, electrosorption, *Escherichia coli*, polarization, water treatment

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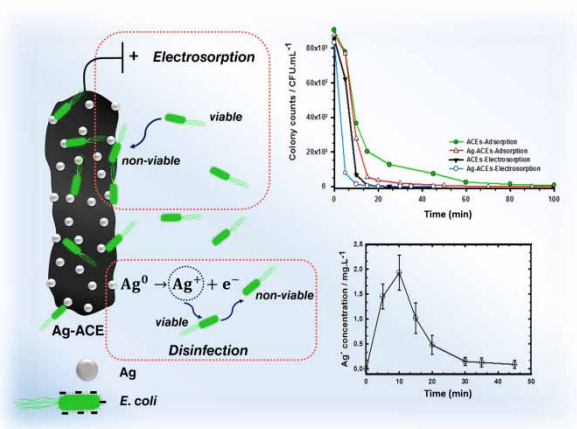
HIGHLIGHTS

- Electrosorption-based scaleable water treatment system has been proposed.
- *E. coli*-containing water can effectively be disinfected by Ag-modified electrodes.
- The polarization of electrodes improves the removal of *E. coli* from aqueous solutions.

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GRAPHICAL ABSTRACT



INTRODUCTION

Contamination of natural and drinking waters by toxic chemicals and bacteria is a significant problem for urban and rural populations. Techniques used in the treatment of wastewaters include coagulation, precipitation, adsorption, membrane filtration, electrochemical techniques, ozonation, chlorination, and fungal decolorization (Hongna *et al.* 2011). Although chemical oxidation with oxidative agents is one of the most effective methods, this method may sometimes produce more toxic by-products than the molecule undergoing oxidation (Kristiana *et al.* 2009). Activated carbon adsorption systems are widely used due to the economic and moderate water treatment efficiencies, especially in the final polishing stage of drinking water treatment plants (Rivera-Utrilla *et al.* 2001). On the other hand, electrosorptive removal techniques, which are defined as adsorption onto potentiostatic or galvanostatic charged electrode surfaces, have been developed as an environmentally friendly technology to increase the adsorption rate and capacity. It offers low cost and high removal efficiency, especially in dilute solutions, with the advantages of electrode regeneration and the potential of complete electrochemical degradation of the adsorbed pollutants. There are many studies on electrosorption of chemical compounds, such as inorganic ions (Oren & Soffer 1983; Ayranci & Conway 2001; Huang & Su 2010), organic molecules (Niu & Conway 2002a, 2002b, 2003; Han *et al.* 2006; Yue *et al.* 2019), pesticides (Ania & Béguin 2007), dyes (Bayram & Ayranci 2010; Bayram *et al.* 2018), and aromatic compounds (Bayram & Ayranci 2012) in literature. Ability and economic benefits in domestic and industrial applications increases the interest in electrosorption using activated carbon electrodes (ACEs). Although there are many works on electrosorption of chemical molecules onto ACE, there are limited works on adsorption or electrosorption of bacteria (Oren *et al.* 1983; Hongna *et al.* 2011; Rivera-Utrilla *et al.* 2001). Oren *et al.* (1983) has investigated the electrosorption of bacteria using porous carbon electrode. In this pioneering study, the authors stated that purification of the suspended bacteria solutions by electrosorption with porous carbon electrodes is feasible and may be advantageous over chlorination and other chemical treatments used for the disinfection of water, because it prevents the medical and environmental effects caused by them. However, increasing the capacity of removing bacteria by electrosorption can lead to the formation of secondary biofilm contamination on the electrode surface (Mossad & Zou 2013). Pandit *et al.* (2017) have systematically studied the possible degradation mechanism of adsorbed microorganisms of electrochemical polarization of carbon aerogel and graphite electrodes. They concluded that the high applied potentials

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may affect the redox potential across the cell membrane and disrupt redox homeostasis, which inhibits the bacterial growth as a result.

This study aimed to remove *Escherichia coli* (*E. coli*) bacteria from aqueous solutions using adsorption and electrosorption onto granular ACEs produced by an industrial method and to prevent *E. coli* growth on the surface. Electroless metal deposition method was used for the modification of activated carbon (AC) granules with silver (Ag) for antibacterial activity. Ag-modified ACEs (Ag–ACEs) were tested for the ability of inhibiting the growth of *E. coli* bacteria, which is commonly found in water. The effect of silver nitrate ($AgNO_3$) concentration on antibacterial activity of Ag–ACEs upon inhibiting the growth of *E. coli* was examined. Adsorption and electrosorption of *E. coli* from aqueous solutions onto ACEs and Ag-ACEs were studied in a cyclic electrosorption system and the capacity of the electrodes was determined.

METHODS

Coconut shell-based granular activated carbon and powder polyethylene were from Sel Corp. Turkey. The granular activated carbons were washed with high purity water until they reached a constant pH and ionic conductivity, dried and then mixed with the polyethylene powder used as a binder in a predetermined amount. After homogenous mixing of the activated carbon granules and binder with 5% pure water, the mixture was heated at 145 °C to melt the polyethylene and subsequently pressed under a pressure of 15×10^6 Pa to fabricate a monolith electrode. An electroless metal deposition method was used for the modification of AC granules with Ag for antibacterial activity. First, 100 g of AC granules were immersed into 250 mL of 0.125 M $AgNO_3$ or 0.250 M $AgNO_3$ solutions in the dark for 24 h and were filtered after equilibration. Ag^+-loaded AC granules were treated with 100 mL of 0.1 M sodium borhydride ($NaHB_4$) solution to reduce Ag^+ to Ag^0. Then AC granules were filtered and washed with deionized water several times until no Ag^+ was left in the filtrate (tested with sodium chloride (NaCl) solution). Pellets were prepared for antibacterial test following same procedure in fabrication of ACEs except a pressure of 40×10^6 Pa was applied. Inductive coupled plasma–mass spectrometry (ICP–MS) measurements were performed to determine the dissolved Ag^+ during the electrosorption of *E. coli* onto Ag–ACEs. Briefly, 1 mL of *E. coli* solution was treated with 6 M nitric acid (HNO_3) and diluted to 20 mL using high purity deionized water and filtered using a 0.22 μm polyvinylidene fluoride membrane. Then, 5 μL of filtrate was injected into an ICP–MS (Thermoscientific, Element 2 ICP–MS). Correlation relations and dilution factors were used to calculate the concentration.

Electrical resistivity and iodine number were used as criteria for characterization of the electrodes. The electrical resistance of the ACEs and Ag–ACEs were measured by placing the electrode (5 cm length × 5 cm width × 2 cm height) on four-point probe cell, which included two gold-coated copper plates under 1×10^6 Pa. Resistance (Ω) was calculated according to Ohm's law from the data obtained by measuring simultaneously the voltage and current of the cell with multimeters (Newport, HHM290/N) connected to the power supply (Gamry, PCl4). The resistivity was calculated from the equation of $r = RA/L$, where r is the resistivity and R is the resistance. A and L are the area and thickness of the electrode, respectively. Iodine numbers of both granular activated carbon particles and monolith electrodes were determined according to the method of ASTM-D4607-94. After boiling a set amount of ground sample with 5% HCl for 30 s, iodine solution was poured onto it in a known concentration and mixed for 5 min. The iodine remaining in the filtrate after filtration was titrated with sodium thiosulfate and the number of iodine was calculated.

The antibacterial activity of pellets was investigated against facultative gram-negative *E. coli* (ATCC No: 23282). Lactose Broth (LB) and Violet Red Bile (VRB) agar solid cultures were prepared after sterilization in an autoclave at 120 °C for 20 min. The solid culture was prepared by dissolving 7 g agar and 9 g LB/VRB in 500 mL of water, which was then poured onto a plastic petri dish for gelation. The disk diffusion method was used to investigate antibacterial activity of the materials (AATCC Test Method 1976; Bayram 2016). Briefly, a colony of *E. coli* was cultivated in 5 mL of LB culture at 37 °C for 24 h and then diluted to the desired volume. These inoculums were incubated for 5.5 h at 37 °C, rotated at 200 rpm (Sayılkan *et al.* 2009),

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and applied to the solid VRB agar medium by dispersing. The AC and Ag-AC pellets were sterilized in an ultraviolet box for 1 h. The pellets were dampened with sterile water, subsequently placed on inoculated VRB agar mediums, and incubated at 37 °C for 12 h. The inhibition zone around the pellets was imaged at the end of the incubation period. The concentration of *E. coli* in its aqueous solutions was determined from the optical density measurements at 540 nm, assuming the optical density of 1.0 is equivalent to approximately 10^9 colony forming units (CFU)/mL. Subsequently, the number of cells was calculated from the standard calibration.

A cycling continuous flow electrosorption was set up for adsorption/electrosorption experiments (Figure 1). The system consists of a peristaltic pump, a solution vessel containing *E.coli* solution on a magnetic stirrer, a potentiostat/galvanostat (Gamry Instruments Inc.) and the flow-through electrolytic cell specially designed for this work.

The flow of ~10^5 CFU/mL *E. coli* solution into the vertically positioned electrosorption cell operating in up-flow-plug mode and then back to the solution vessel was carried out using a peristaltic pump and silicon tubing (0.2 cm internal diameter). A magnetic stirrer was used to homogenize the mixture. Polarization of the electrodes was achieved using a potentiostat/galvanostat. ACEs or Ag–ACEs were used in the electrosorption cell as symmetrical cathode and anode electrodes. The total weight of the cathode and anode was 50 ± 2 g. The concentration of *E. coli* in solution was determined by inoculation of 1 mL solution into VRB agar solid medium at certain time intervals during the cycling and counting the colonies using a colony counter after 24 h incubation at 37 °C.

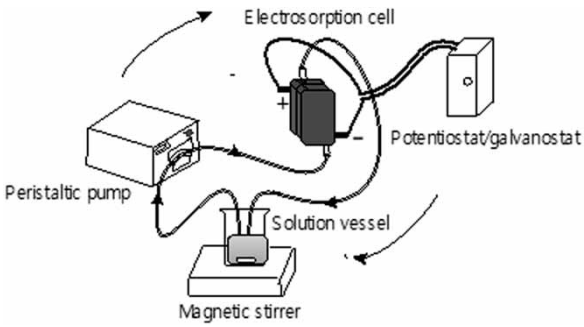


Figure 1 | Experimental set-up of continuous flow electrosorption process.

RESULTS AND DISCUSSION

Fabrication and characterization of ACEs

The washed and dried AC particles were first sieved to have a size of 350–500 μm for the standard manufacturing process. They were then moistened with 5% pure water to ensure that the binder polyethylene powders adhere to the AC surface, thereby making the mixture homogeneous. The addition of water to the mixture also allowed the mixture to be heated to 145 °C rapidly and homogenously in the microwave oven and to press the molten polyethylene without diffusing into the AC pores. As a result, as the AC pores were not clogged, a higher surface area with a lower polyethylene ratio was obtained. Preliminary tests were performed for determining certain amount of polyethylene in ACEs (not given). AC granules were mixed with polyethylene in the ratio of 5%, 15%, 35% and 50% (w/w). In higher ratios of polyethylene the conductivity and iodine number value of AC electrodes were found to be decreased, whereas, lower ratios decreased the structural integrity. Thus, 15% of polyethylene ratio was selected for the fabrication of ACEs on the basis of resistivity, iodine number, and structural integrity. Images, taken from different magnitudes of ACEs prepared from 15% polyethylene and 85% AC, are shown in Figure 2.

Monolithic electrode structure consisting of AC granules successfully adhered with polyethylene powders can be seen in Figure 2(a) and 2(b). The scanning electron microscopy–energy-dispersive X-ray spectroscopy (SEM-EDS) images recorded on a ZEISS EVO MA10 at 20 kV given in Figure 2(c) clearly show the successfully deposited micron-sized Ag particles on AC. In electrochemical processes, the electrical conductivity due to the resistivity of the electrode is vital for the effectiveness of the process. The resistivity of ACEs and Ag–ACEs were calculated as 0.87 ± 0.18 Ω·m and 0.65 ± 0.12 Ω·m, respectively. This value is comparable with activated carbon fibers given in literature (Johnsen *et al.* 2014) and allows homogeneous distribution of applied potential or current on the electrodes used for the electrosorption process. The strong adhesion between AC granules and homogeneously distributed polyethylene allowed the electric current to be effectively dispersed along the electrode. The decrease in resistivity (increase in conductivity)

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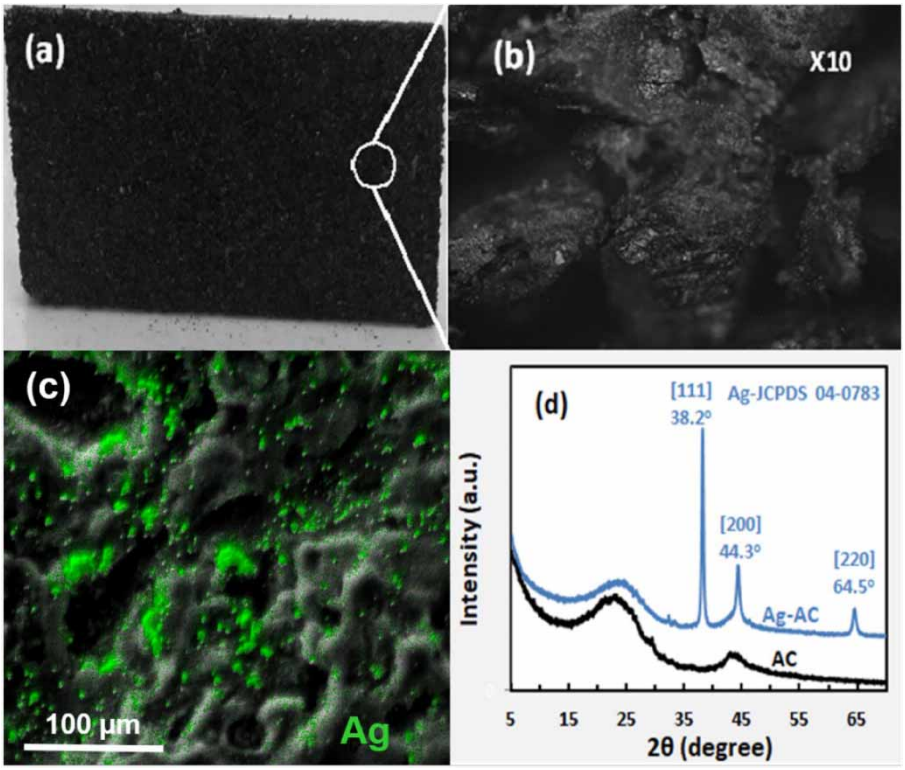


Figure 2 | (a), (b) Images of ACEs taken at different magnifications. (c) Elemental mapping of Ag particles deposited on AC (Ag-AC) granules obtained by SEM-EDS analysis at ×1,000 magnification (green spots represents Ag). (d) XRD pattern of AC and Ag-AC granules. Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/ws.2020.282.

from ACEs to Ag–ACEs is probably due to the Ag particles incorporated on to the AC granules.

The effectiveness of the electrosorption process is also directly related to the surface area of the electrodes used. The iodine number can be used to estimate the surface area of activated carbons at room temperature conditions (Nunes & Guerreiro 2011). In this study, to obtain reliable surface area values, iodine numbers, rather than the N_2 adsorption method (which uses a homogenous sample in milligrams), were used for the electrodes because of the difficulties in grinding polyethylene as a binder.

Iodine numbers of pristine granular activated carbon particles, ACEs, and Ag–ACEs were calculated as 1,062 $mg{\cdot}g^{-1}$, and 840 $mg{\cdot}g^{-1}$, and 803 $mg{\cdot}g^{-1}$, respectively. The decrease observed in iodine numbers from pristine granular activated carbon particles to ACEs and Ag–ACEs is because the polyethylene particles and/or Ag particles were blocked from entering the pores. Although a decrease in iodine number was observed, ACEs and Ag–ACEs still have an acceptable surface area required for an effective electrosorption process (Nunes & Guerreiro 2011).

Antibacterial activity

The AC electrodes were loaded by Ag particles to prevent the formation of biofilm on the AC electrodes. Loading of Ag particles were performed on granular AC particles in situ. First, AC particles dipped into $AgNO_3$ solutions and Ag^+ ions were adsorbed on to AC particles, and the ions were reduced to Ag^0 where they adsorbed. Antibacterial activity of Ag-loaded AC particles were tested against *E. coli* bacteria and the results are shown in Figure 3.

Formation of a clear zone around pelletized Ag-AC confirms the antibacterial activity. However, the effect of $AgNO_3$ concentration on antibacterial activity of AC is not clear because the zone diameters are almost identical. Thus, electrodes prepared from 0.125 M $AgNO_3$ solution

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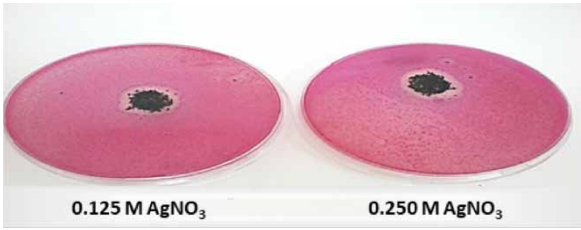


Figure 3 | Antibacterial activity test results of Ag-loaded AC pellets prepared from 0.125 M $AgNO_3$ and 0.250 M $AgNO_3$ solutions.

were used in adsorption and electrosorption experiments as Ag-modified electrodes (Ag–ACEs).

Adsorption/electrosorption of *E. coli*

Adsorption and electrosorption experiments of *E. coli* onto ACEs and Ag–ACEs were performed using the system given in Figure 1. The 500 mL bacteria solution was pumped through the cell by a peristaltic pump with a constant volumetric flow rate of 60 mL·min^{-1} throughout a loop. *E. coli* concentration values obtained from the samples taken at certain time intervals during cycling according to the method described earlier are shown in Figure 4(a).

A sharp decrease in the number of bacteria in aqueous solution was observed in first 15 min in all cases. The adsorption of *E.coli* onto Ag-loaded ACEs seems to be more favorable than onto the ACEs, probably because of the growth inhibition effect of Ag on Ag–ACEs where both Ag^0 and Ag^+ have an antibacterial effect against *E. coli* (Choi *et al.* 2018). Thus, the differences in ACEs and Ag–ACEs processes should not be evaluated solely in terms of the amount of *E. coli* adsorbed onto the surface of the electrodes. This difference is the sum of the amount of *E. coli* removed and growth-inhibited *E. coli* in solution by Ag particles. Therefore, *E. coli* removal and growth inhibition in solution are performed simultaneously in adsorption and electrosorption processes in the case of Ag–ACEs. However, after even 100 min of the adsorption process, some colonies were still observed in solutions of both ACEs and Ag–ACEs. Electrosorption process of *E.coli* was conducted by polarization of ACEs and Ag–ACEs by applying 1.5 V constant potential. It is to be noted that the reason for applying the 1.5 V potential originates from our previous findings that showed this to be the upper limit for polarization of activated carbon

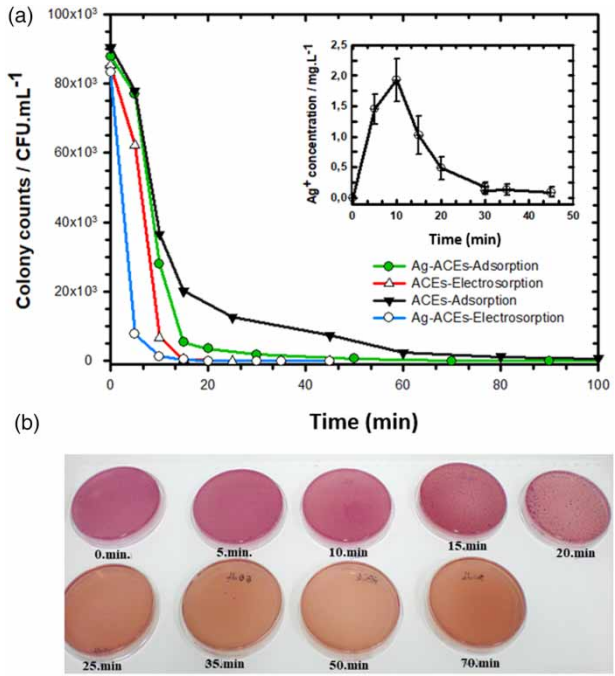


Figure 4 | (a) Change in concentration of *E. coli* during the adsorption and electrosorption onto ACEs and Ag–ACEs (inset shows *ex situ* concentration measurement results of Ag^+ in *E. coli* solution during the course of electrosorption onto Ag–ACEs). (b) Petri dishes inoculated with *E. coli* solutions sampled at different time intervals during electrosorption with Ag-ACEs at 1.5 V polarization after 24-h incubation.

electrode in aqueous solutions free from any Faradaic reactions (Bayram & Ayranci 2010; Bayram *et al.* 2018). It is obvious that the polarization of electrodes improved the removal efficiency of *E.coli* from aqueous solution onto both ACEs and Ag–ACEs. In the case of electrosorption with Ag–ACEs, the electrosorption rate seems to be much higher than the electrosorption with ACEs, and almost complete disinfection of *E. coli* was achieved from 500 mL of ~10^5 CFU/mL solution by Ag–ACEs via electrosorption process within 30 min. Polarization effects on removal of *E. coli* arise from the electrostatic attraction between the negatively charged *E. coli* cell membrane (Jastrzębska *et al.* 2017) and positively charged ACEs. Additionally, growth inhibition of the *E. coli* cell membrane by Ag^+ ions is responsible for the reduction of *E. coli* in the first 30 min, as in the case of adsorption onto Ag–ACEs. Diffusion of Ag^+ ions into solution as a result of possible electrochemical oxidation during electrosorption was also checked by *ex situ* ICP–MS measurements (inset, Figure 4(a)). A sharp increase in Ag^+ concentration was observed due to the electrochemical

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oxidation of Ag in the first 10 min of the process. However, probably due to the sorption of the Ag^+ by *E. coli* in the adsorbed state and/or reduction at the cathode, the concentration of Ag^+ decreased rapidly within the next 10 min and approached zero at about the 50th min of the process. This result explains the higher electrosorption efficiency of Ag–ACEs than the ACEs. Figure 4(b) shows the petri dishes inoculated with *E. coli* solution at different times by sampled during electrosorption onto Ag–ACEs at 1.5 V polarization after 24 h of incubation which are. It should be noted that VRB is a specific indicator for *E. coli*. in the absence of *E. coli* its color turns red to orange. The color of dishes after 35 min of electosorption process visually confirms the presence of *E. coli* in the medium.

The formation of biofilm on the electrodes used in the electrosorption process was tested by placing 0.5 g of ACEs and Ag–ACEs particles in VRB agar medium and incubating at 37 °C for 12 h. The images of petri dishes incubated with used electrode pieces are shown in Figure 5.

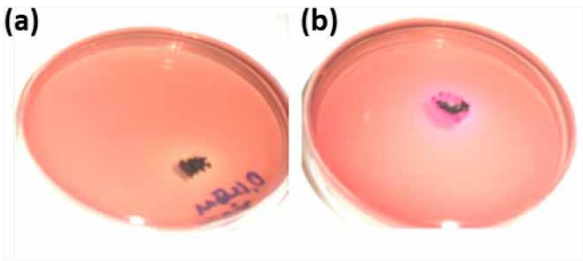


Figure 5 | Images of petri dishes after 24 h incubation of (a) Ag–ACEs and (b) ACEs particles used in electrosorption of *E. coli*.

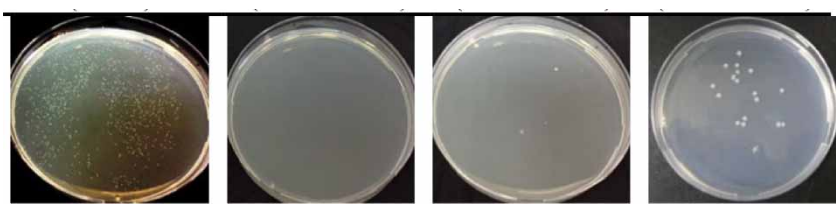
The absence of *E. coli* colonies on the VRB agar medium indicated the antibacterial activity of Ag–ACEs electrodes (Figure 5(a)). However, red spots appearing around the ACEs indicated the presence of viable *E. coli* and formation of biofilm on AC particles (Figure 5(b)). The presence of viable *E. coli* on ACEs confirms the non-Faradaic nature of the electrosorption process. This is an important advantage for electrosorption because electrooxidation/reduction by-products are more toxic than *E. coli* (Simon *et al.* 2018) due to the complex molecular composition of microorganisms.

Capacity of the electrosorption system

The capacity of a system for a given pollutant is an important parameter for practical applications. For this purpose, a series of successive electrosorption experiments were performed by cycling fresh portions of 500 mL of 1.1×10^5 CFU·mL^{-1} *E. coli* solutions for 35 min at a volumetric flow rate of 60 mL·min^{-1} and 1.5 V polarization without changing the Ag–ACEs electrodes. Then 1.0 mL of samples were taken from the reservoir at the beginning (0 min) and after 35 min of each experiment to determine the difference in the number of bacteria. Table 1 shows of the petri dishes inoculated at certain time intervals with the treated solution and the performance parameters calculated from CFU on the petri dishes, taking into account the dilution factors. Each white point in the images represents a colony forming unit.

Complete removal of *E. coli* from 1,000 mL of 1.1×10^5 CFU·mL^{-1} solution was achieved, with >99% removal

Table 1 | Images of petri dishes, inoculated with treated solution sample at certain time intervals and the calculated removal efficiency values

	Control (0 min)	Portion 1 (After 35 min)	Portion 2 (After 35 min)	Portion 3 (After 35 min)
Images of *E. coli*-inoculated petri dishes				
Colony count (CFU·mL^{-1})	1.1×10^5	<10	< 10	1.5×10^3
Removal efficiency (%)	0.00	99.99	99.99	98.64

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efficiency after two successive electrosorption processes. After the third process (Portion 3), white spots appeared, showing that >98% removal efficiency was reached. This probably indicates that the Ag on the anode was depleted as a result of oxidation (inset of Figure 4(a)), and the cathode and anode have reached capacity. In summary, the applicability of ACEs and Ag–ACEs for electrosorption of *E. coli* has been demonstrated in this study. Our future studies will be focused on the optimization of system parameters such as applied potential and volumetric flow rate, and will be extended to bacteria species other than *E. coli*.

CONCLUSIONS

An industrial process was applied for fabrication of ACEs and Ag–ACEs. It was found that *E. coli* removal efficiency of Ag–ACEs prepared by using 0.125 M $AgNO_3$ are better than ACEs, and polarization of Ag–ACEs could enhance the removal efficiency of *E. coli* from its aqueous solutions. In addition, the formation of biofilm on AC electrodes was prevented by modification of AC electrodes with Ag.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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