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Removal of *Escherichia coli* bacteria from drinking water by dielectrophoresis

Jinxin Liu[a], Dongyang Liu[a], Junfeng Geng[b], Jianxin Xia[a], Huiying Chen[a,*], Yanhong Wu[a,*]

[a]*College of Life and Environmental Sciences, Minzu University of China, 100081 Beijing, China, Tel. +86-13910159837; email: huiyingrchen@aliyun.com (H.Y. Chen), Tel. +86-13801232755; email: 9900547@muc.edu.cn (Y.H. Wu), Tel. +86-18180346599; email: 2281445042@qq.com (J.X. Liu), Tel. +86-18811594623; email: dongyangliu@whu.edu.cn (D.Y. Liu), Tel. +86-010-68933621; email: jxxia@vip.sina.com (J.X. Xia)*

[b]*Institute for Materials Research and Innovation, Institute for Renewable Energy and Environmental Technologies, University of Bolton, Deane Road, Bolton, BL3 5AB, U.K., Tel. +44 (0) 1204903842; Fax +44 (0) 1204900600; email: j.geng@bolton.ac.uk (J.F. Geng)*

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ABSTRACT

Working on a dielectrophoresis experimental system assembled in our laboratory, we find that the bacteria, *Escherichia coli*, can be efficiently removed from drinking water by the system. Synthetic water samples of 300 mL of *E. coli* were treated for 20 min at a flow rate of 1 L/h. The removal efficiency reaches 95.24% high at the voltage of 6 V, and 100% at 15 V. As the technique is featured by its simplicity and low cost for water treatment, it promises a rapid, affordable, and industry-scale removal of *E. coli* and other similar pathogenic microorganisms from drinking water.

Keywords: Drinking water; *Escherichia coli*; Dielectrophoresis

1. Introduction

The existence of bacteria in drinking water has been a major public health concern as data have shown that about 80% of communicable diseases are waterborne worldwide [1]. This issue is particularly problematic in developing countries, but even in developed countries with strict management of drinking water, there are still risks of waterborne bacterial diseases originating from piped water supply [2,3]. Among many types of bacteria, *Escherichia coli* is a distinct indicator of fecal contamination in water [4,5]. Although most *E. coli* strains do not cause disease, virulent strains can cause gastroenteritis, urinary tract infections, neonatal meningitis, hemorrhagic colitis, and Crohn's disease with severe symptoms such as diarrhea, nausea, headache, fever, vomiting, etc. [6–8]. Children are more susceptible to develop severe illness, however, healthy individuals of all ages are at risk to the severe consequences that may arise from infection with *E. coli*.

E. coli encompasses an enormous population of bacteria that exhibit a very high degree of both genetic and phenotypic diversity, and they widely exist in rivers and springs [9]. Up to 2004, there are about 5 million people who still obtain water from rivers and springs with excessive amount of harmful bacteria including *E. coli* in South Africa [10]. Studies have shown that the majority of small water plants in South Africa have difficulty in providing adequate treatment and disinfection to drinking water [11]. In remote villages of China, mountain spring has been used as drinking water by local people, which contains *E. coli* [12]. It has been reported that worldwide, drinking waterborne pathogens including *E. coli*, kills more than 2.5 million people a year [13]. A number of *E. coli* strains, for example, *E. coli* O157:H7, produce a powerful toxin and can cause severe illness to healthy humans and animals when they use a drinking water source like rivers and mountain springs [8,10,14]. Therefore, it is no doubt that the effective removal of *E. coli* from drinking water to reduce the morbidity of waterborne diseases is highly demanded.

* Corresponding authors.

Traditional methods used to remove *E. coli* from water are mainly concerned with membrane separation [15], adsorption [16], chlorination [17], ultraviolet (UV) radiation [18], slow sand filtration [19] and ozone disinfection [20]. However, these methods suffer from problems of either high cost, low efficiency, or possible contamination.

Dielectrophoresis (DEP) refers to the directional migration of particles subjected to dielectric polarization in a non-uniform electric field. Nevertheless, the applications of DEP in the separation of living cells [21], and in the removal of heavy metal ions from wastewater [22,23], are well documented. We would like to propose the following mechanism to explain the DEP process at cellular level: a non-uniform electric field generated in the DEP device polarizes *E. coli* cells and induces a dipole moment on each of them. As a consequence, the electric field exerts an unbalanced force on the rod-like *E. coli* cells, which drives them to move along the electric field gradient in the solution. Those cells near the electrodes were first captured and trapped by the electrodes. Due to the strong electric field in the cross-wire areas of the mesh electrodes, coupled with a possible polarization induction effect between adjacent cells, other *E. coli* cells close to the wire junctions were polarized and subsequently trapped by the electrodes. In this way, continuous capture of the cells would occur, so more and more *E. coli* would be trapped and deposited on the electrodes (Fig. 1). This mechanism is supported by the working principle of DEP – a technique that has been used to manipulate polarized particles suspended in fluid media in non-uniform electric field [24]. In the case of a spherical particle, the dielectrophoretic force F_{DEP} is given by the equation below [25]:

$$F_{DEP} = 2\pi R^3 \varepsilon_m \mathrm{Re}\left[K(\omega)\right]\nabla E^2 \quad (1)$$

where the real part of Clausius–Mossotti factor, Re[$K(\omega)$], is defined as:

$$K(\omega) = \frac{\varepsilon_p^* - \varepsilon_m^*}{\varepsilon_p^* - 2\varepsilon_m^*} \quad (2)$$

where R denotes the radius of the particle, ∇E the magnitude of the electric field gradient, ε^*_p the complex permittivity of the particle, and ε^*_m that of the media. A non-uniform electric field is necessary to induce a DEP force as stated in Eq. (1) (otherwise $\nabla E = 0$). Positive values of Re[$K(\omega)$] denote the induction of a positive DEP force that causes particles to be trapped in the regions of high electric field gradient. Negative values of Re[$K(\omega)$] denote negative DEP, which means the particles would move towards the regions of low or no electric field. As all the *E. coli* cells were trapped on the cross-wire areas of electrodes where the electric filed gradient was the strongest, indicating that positive DEP force was generated in this work.

Here we report an exceptionally efficient DEP based method to directly remove *E. coli*, a representative bacteria, from drinking water. The study presents a new method with potential to remove other harmful microorganisms. With the technique we can currently treat *E. coli* contaminated water in a flowing volume of 1 L/h in the laboratory. In addition, we elucidate the individual roles played by the processing factors such as the DEP operation time, voltage, and initial *E. coli* concentration.

2. Material and methods

2.1. Materials

The wire mesh electrodes were made by 316 stainless steel, and the space between each weir was 200 μm. Direct current power device (PS-305DM, Longwei Instruments (HK) Co., Ltd., China) was used as the power source. Untreated drinking water was sampled from Dulongjiang River, a remote mountain village of Yunnan Province, China. Synthetic water samples were prepared using distilled water and DH5α *E. coli* at a concentration of 2,000 CFU/mL and diluted to different orders of magnitude of concentration for exploring optimal conditions. 3M Petrifilm™ of *E. coli* (Minnesota Mining and Manufacturing Co., USA) was used to measure the concentration of *E. coli*.

2.2. Methods

Our DEP experiments were conducted with a home-constructed apparatus shown in Fig. 2. A series of 300 mL of *E. coli* solutions with an initial concentration of 1,000 CFU/mL (except the experiment for effects of initial concentration) were fed from a conical flask into a fluidic vessel via a

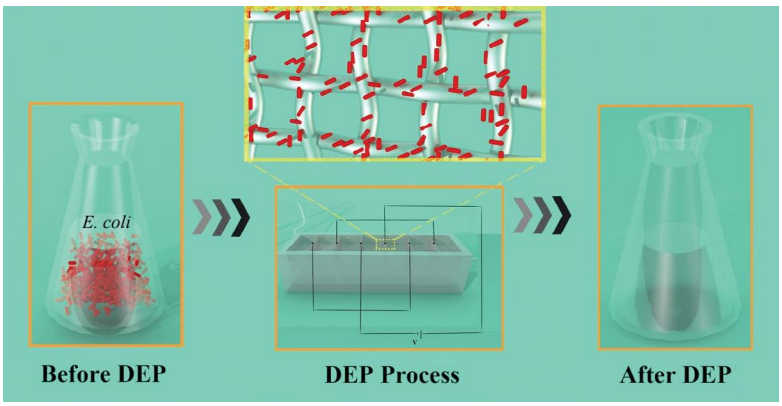

Fig. 1. Schematic diagram shows the capture and removal process of *E. coli* by the DEP method.

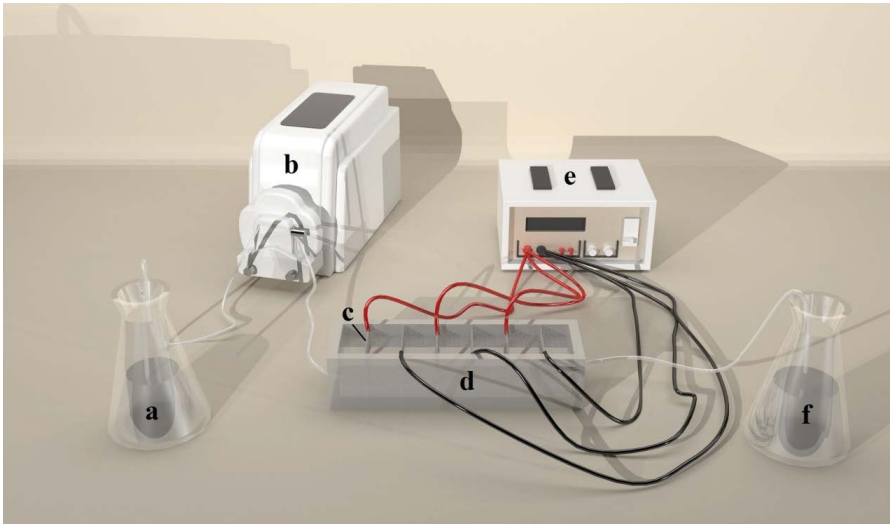

Fig. 2. Schematic of the DEP processing device. (a) Conical flask for fluid storage; (b) mechanical pump; (c) 316 stainless steel wire mesh electrodes; (d) fluid vessel; (e) direct current power source; (f) fluid reception flask.

mechanical pump. The 10 electrodes, with each containing 316 stainless steel wires, were installed at the slots on both sides of the fluid vessel. These electrodes were separated by a fixed distance of 10 mm between two adjacent ones. The voltages were supplied by a direct-current power device. Batch experiments were carried out in the voltage range of 3–15 V, with DEP processing times of 20 min (except for the experiment for effects of processing time).

The mesh electrodes were removed from the vessel when the experiment was finished, and the deposited *E. coli* were washed off the electrodes by distilled water. The concentrations were measured before and after each DEP treatment by the Petrifilm™ method [26]. In specific, 1 mL water sample was first added into an *E. coli* counting plate, and the cultured colonies were counted after 24 h to determine the concentration.

3. Results and discussion

3.1. Effect of the DEP related factors

3.1.1. Effect of voltage

A characteristic feature of using DEP to remove pollutants from the liquid is that the removal efficiency is voltage related. The voltage applied to the electrodes will directly affect the intensity of the non-uniform electric field which is proportional to the DEP force on the suspended particles. The effects of voltage in the range of 3–15 V on the removal effect were investigated at an initial *E. coli* concentration of 1,000 CFU/mL, a flow rate of 1 L/h, and a treatment time of 20 min.

It was observed that in our case of removing *E. coli* from water, even a low voltage could have a remarkable effect on the bacteria. Fig. 3a shows that when a voltage of only 3 V was applied to the electrodes, the removal efficiency reached ~81% in 20 min, and when the voltage was 6 V, even higher removal efficiency of 87.6% was gained. It was also observed that when the voltage was up to 15 V, all the *E. coli* were completely removed from the water so a 100% removal efficiency was achieved. Having said that, we chose to use lower voltages in the experiment as high voltage could have a risk of causing the electrolysis of the stainless steel electrodes and bubbling of water, which would reduce the lifetime of the device. Martinez-Duarte [27] also point out that metal electrodes can interact with the samples at high voltages, thus reducing the lifetime of the electrodes. It is worth noting that we used a DC power supply with a maximum experimental voltage of 15 V, which is safe and easily accessible. Considering operating costs, safety, and removal rates, we chose to fix the voltage at 6 V in the following experiments to investigate the effects of other processing factors on removal efficiency.

3.1.2. Effect of processing time

Synthetic water samples were treated at an initial concentration of 1,000 CFU/mL with a flow rate of 1 L/h and a voltage of 6 V. Samples were taken at 5-min intervals and the effect of treatment time on the removal of *E. coli* was investigated. Fig. 3b shows how the processing time affected the removal efficiency. It can be seen that with an increase of the processing time, the removal efficiency went up rapidly at the beginning but slowed down in about 15 min. At the 20 min moment, the efficiency reached the value of 87.6%, which may be compared with the data of 50% obtained by Dunlop et al. [28] in 60 min where they used a photo-catalysis method to remove *E. coli* from water.

3.1.3. Effect of initial concentration

In addition, we investigated the removal of *E. coli* at different concentrations by setting the voltage to 6 V with a treatment time of 20 min and a flow rate of 1 L/h, and the result is shown in Fig. 3c. With a fixed voltage and processing time, the removal efficiency increased with the decrease of the initial concentration, as it was expected. Interestingly, even at high initial concentrations (such as 2,000 CFU/mL in this case), which have reached the high contamination

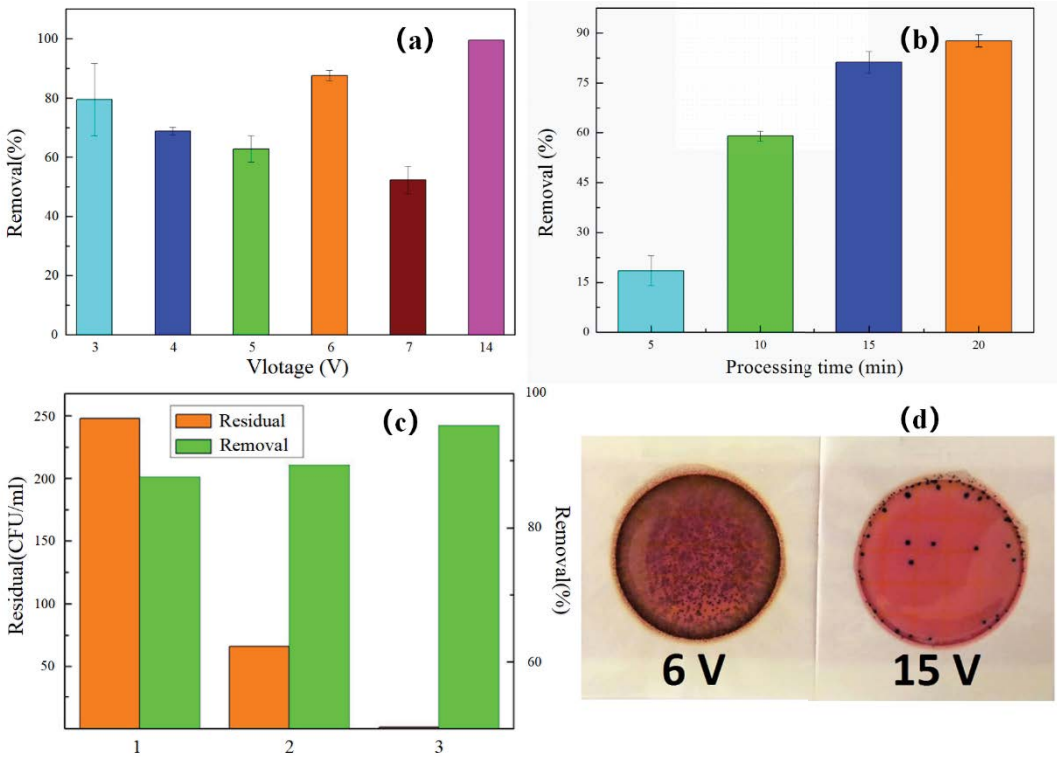

Fig. 3. (a) The removal efficiency of *E. coli* at different voltages in 20 min when the initial concentration was 1,000 CFU/mL, (b) The removal efficiency at different processing times with a fixed voltage of 6 V and initial *E. coli* concentration of 1,000 CFU/mL, (c) A diagram shows the removal of *E. coli* at different initial concentrations after 20 min. From column 1 to 3, the initial concentration was 2,000, 618, and 21 CFU/mL, respectively, and (d) images show the activity of *E. coli* trapped on the DEP electrodes at different voltages. In this case, the initial concentration of *E. coli* was ~10^5 CFU/mL.

levels of *E. coli* presented by the World Health Organization [29,30], the removal efficiency of our DEP method remains high (~87.4%). It can also be seen from Fig. 3c that when the initial concentration was down to 21 CFU/mL, a level which may be compared with the normally low concentrations of *E. coli* encountered in rivers or springs, the removal efficiency was up to 95.24%, and this was achieved in only 20 min operation at a low electrode voltage of 6 V. When we increased the voltage to 15 V and kept the initial concentration low (e.g., 92 CFU/mL), it was observed that a 100% removal efficiency could be easily achieved in 20 min, and the results is shown in Table S2.

3.2. Activity of E. coli under different voltages

It is worth noting that the voltage applied to the electrodes could also affect the activity of the *E. coli* cells trapped on the electrodes. The *E. coli* cells were first trapped in the electrodes at 6 and 15 V, respectively. After being captured, the cells were collected by washing the electrodes with distilled water, and then cultured for 24 h. The activity of *E. coli* cells was observed by microscope, as shown in Fig. 3d. It could be seen that there were a large number of active *E. coli* cells in the sample trapped at 6 V. In contrast, very few *E. coli* cells could be seen in the sample collected at 15 V. This indicates that *E. coli* cells are basically alive at 6 V, but hardly survive at 15 V.

It was considered that the inactivation of *E. coli* cells captured at 15 V may be attributed to the higher electric current density, 114 mA/cm^2, with which most *E. coli* cells were killed. This view was consistent with that of Jeong et al. who investigated the effect of the anodic current density on *E. coli* inactivation during the electrochemical disinfection process using Pt anode. And they concluded that *E. coli* could be killed when the electric current density was 100 mA/cm^2. In their work, the maximum inactivation of *E. coli* was 90% after 180 min in 0.2 M KH_2PO_4 solution. In comparison, our DEP experiment showed a higher removal efficiency in a shorter time without the addition of KH_2PO_4 into the solution, which can not only save treating time but also reduce secondary pollution caused by chemical reagents.

3.3. Feasibility of practical applications

Lapizco-Encinas et al. [31] a method for the direct capture of bacteria, spores, and viruses in water by insulator-based DEP (iDEP), which showed that particles are reversibly captured in the DEP device when the applied electric field is above a specific threshold for the particles. Syed et al. [32] used a photolithographic process to prepare a nano DEP device to enable the capture and detection of *E. coli*, which demonstrated that DEP can guide the flow of *E. coli* in a microfluidic channel. The possibility of *E. coli* capture

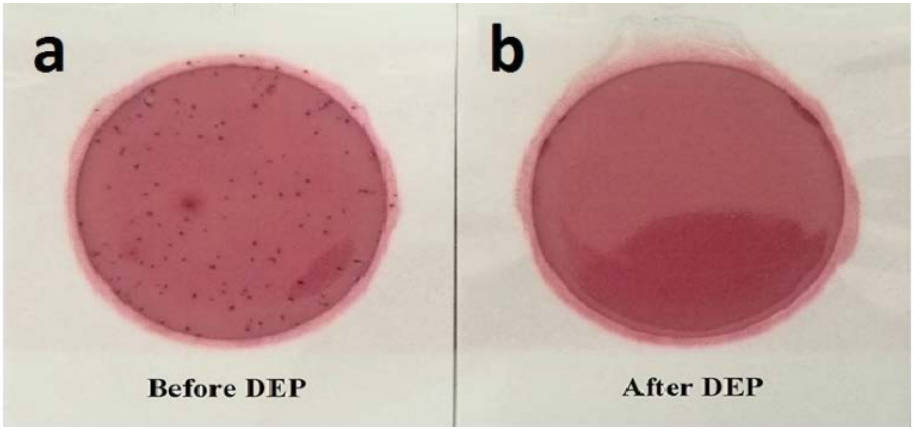

Fig. 4. (a) Image of untreated drinking water containing *E. coli* with a concentration of ~100 CFU/mL and (b) image of the same sample after the DEP treatment at 15 V for 5 min to show a complete removal of the bacteria.

by DEP has been demonstrated and we hope to be able to apply this technique to the treatment of real drinking water. Therefore, we treated field-obtained water samples from the Dulong River (initial concentration 10^2 CFU/mL) with DEP. After 5 min of treatment at a flow rate of 1 L/h and a voltage of 15 V, no active *E. coli* colonies were present in the water. The results are shown in Fig. 4.

Since our DEP device could be enlarged in size, based on the same assembling principle, it could be potentially used to treat much larger volumes of *E. coli* contaminated drinking water for practical and domestic applications. However, the detailed processing mechanism as regards to why the DEP is so effective in removing *E. coli* from water is still poorly understood at the moment, suggesting that extensive future research work is needed to fully explain the removal process at molecular and atomic levels.

4. Conclusion and recommendations

We have made a DEP device and used it to experiment with the removal of *E. coli* from drinking water. We find that the method is efficient for the purpose as demonstrated by the high removal efficiencies of 95.24% at 6 V and 100% at 15 V for the synthetic water samples, in a short operation time of 20 min, respectively, at a treatment volume of 1 L/h. Moreover, we notice that the *E. coli* trapped on the DEP electrodes can be directly inactivated by the electrodes when the voltage was sufficiently high, whereas at lower voltages it is mainly captured directly by DEP. The direct current power device used for the experiments is commercially available and the overall equipment is simple and easy to maintain and manage. Our DEP experiments are also very cheap to run and safe as high removal rates can be achieved at lower voltages. However, due to practical constraints, it is currently limited to the laboratory. In the future, we will focus on scaling up the experiment to treat high flows of water. And for higher concentrations of *E. coli*, we can connect more DEP treatment equipment in series to improve removal efficiency and reduce the voltage. In addition, cleaning equipment should be added to regularly remove captured contaminants to reduce the potential for secondary contamination.

Considering these experimental results, and the potential that our DEP device could be enlarged in size to treat *E. coli* contaminated water at a larger scale, we believe that the approach demonstrated in this work represents a potentially significant step forward towards a rapid, viable, and industry-scale solution to meet the high demand of removing *E. coli* from drinking water in many places. This could be particularly beneficial for people living in developing countries to obtain clean drinking water in their daily lives.

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Conflict of interest

The authors declare no conflict of interest.

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Supplementary information

S1. Experimental

The treatment of the *E. coli*-containing water by dielectrophoresis (DEP) was carried out at room temperature. The electrodes were installed at the slots on both sides of the fluid vessel. The distance was set at 10 mm between two adjacent electrodes. The voltages were supplied by a direct current power device. When untreated water flowed through the DEP apparatus, the *E. coli* bacteria were trapped on the electrodes. The water samples were treated in this way under different processing time, voltage and initial concentration by DEP to investigate the effects of these operation parameters on the removal efficiency. The concentration of *E. coli* was measured before and after each DEP treatment. To do this, 1 mL water sample was added into a *E. coli* count plate, and the cultured colonies were counted after 24 h to determine *E. coli* concentration (Petrifilm™ method).

The verification of activity of *E. coli* in different voltages was carried out with the same level initial concentration following a 20 min treatment by DEP. *E. coli* trapped on the electrodes were washed down by 50 mL distilled water. *E. coli* was measured with the Petrifilm™ method to evaluate its activity.

S2. Removal tests at different voltages

In addition to the data provided in the text, more removal tests were carried out at different voltages but under a lower initial *E. coli* concentration (in this case, 100 CFU/mL). The result is summarized in Table S1.

It can be seen from the table that the voltage of 6 V shows the best result which is consistent with the data shown in the text. At this voltage, the change of the removal

Table S1
Effect of the electric field strength on the removal efficiency

Voltage (V)	5	6	7	9	15
Removal (%)	76.36	89.32	69.5	78.52	100

Fig. S1. Images of cultivated *E. coli* from the water simple after DEP treatment at 6 V.

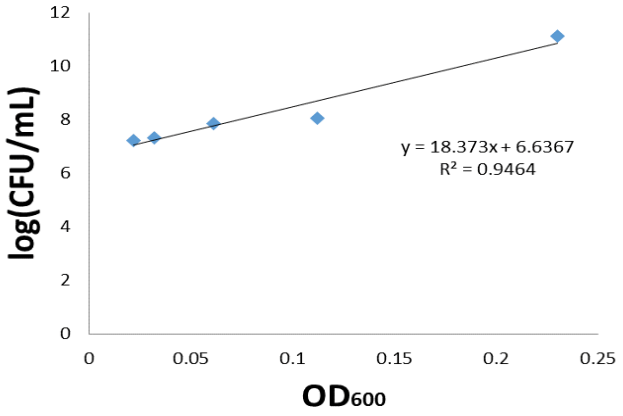

Fig. S2. Calibration of the concentration of *E. coli* vs. its optical density at wavelength 600 nm.

efficiency with time can be visualized from the following images (Fig. S1), where 6V-0 denotes to the time of 0 min, 6V-1 to 5 min, 6V-2 to 10 min, 6V-4 to 20 min.

S3. OD(600) calibration curve and the E. coli concentrations

OD(600) is an abbreviation indicating the absorbance or optical density of a sample measured at a wavelength of 600 nm with UV-Vis Spectroscopy. To do OD(600) calibration curve, the initial solution of *E. coli* was diluted to a concentration with OD(600) at about 0.2. This solution was further diluted by a volume of 2, 4, 8, and 10, with OD(600) value measured against each of such a diluted solution. These five solutions were then added into five *E. coli* count plates, the cultured colonies were counted after 24 h (Petrifilm™ method). Finally, we plot the log(CFU/mL) as a function of OD(600) to obtain the calibration curve (Fig. S2), which would be later used to determine the concentrations of *E. coli* samples.

To determine the concentration of the *E. coli* simple solutions, a certain amount of cultured *E. coli* simple was first taken and its solution was diluted to a OD(600) value of 0.05–0.15. The exact concentration of the solution can be calculated by using the calibration curve. This solution would be further diluted to the concentration required for subsequent DEP test.

S4. Tests of removal efficiency vs. voltage and processing time

In addition to the data provided in the text, more tests were carried out on the voltage and processing time in order

Table S2
Removal efficiency (Unit: %) vs. the voltage (V) and processing time (min). For the test at 14, 15, and 16 V, the corresponding initial concentration was 92, 379 and 131 CFU/mL, respectively

	14 V	15 V	16 V
0 min	0	0	0
5 min	63.04	37.47	92.37
10 min	98.91	94.46	100
15 min	100	99.00	100
20 min	100	100	100

to achieve a 100% removal efficiency. The resulting data were summarized in ESI Table S2. It is clear that if the initial *E. coli* concentration was not more than 379 CFU/mL, all the bacteria could be completely removed from water in 20 min as long as the applied voltage was up to 15 V. When the initial concentration was down to the level of ~100 CFU/mL, then only about 10 min was required to achieve a 100% removal. ESI Fig. S3 shows the corresponding images of the bacteria before and after the DEP treatment.

92	379	131
34	237	10
1	21	0
0	4	0

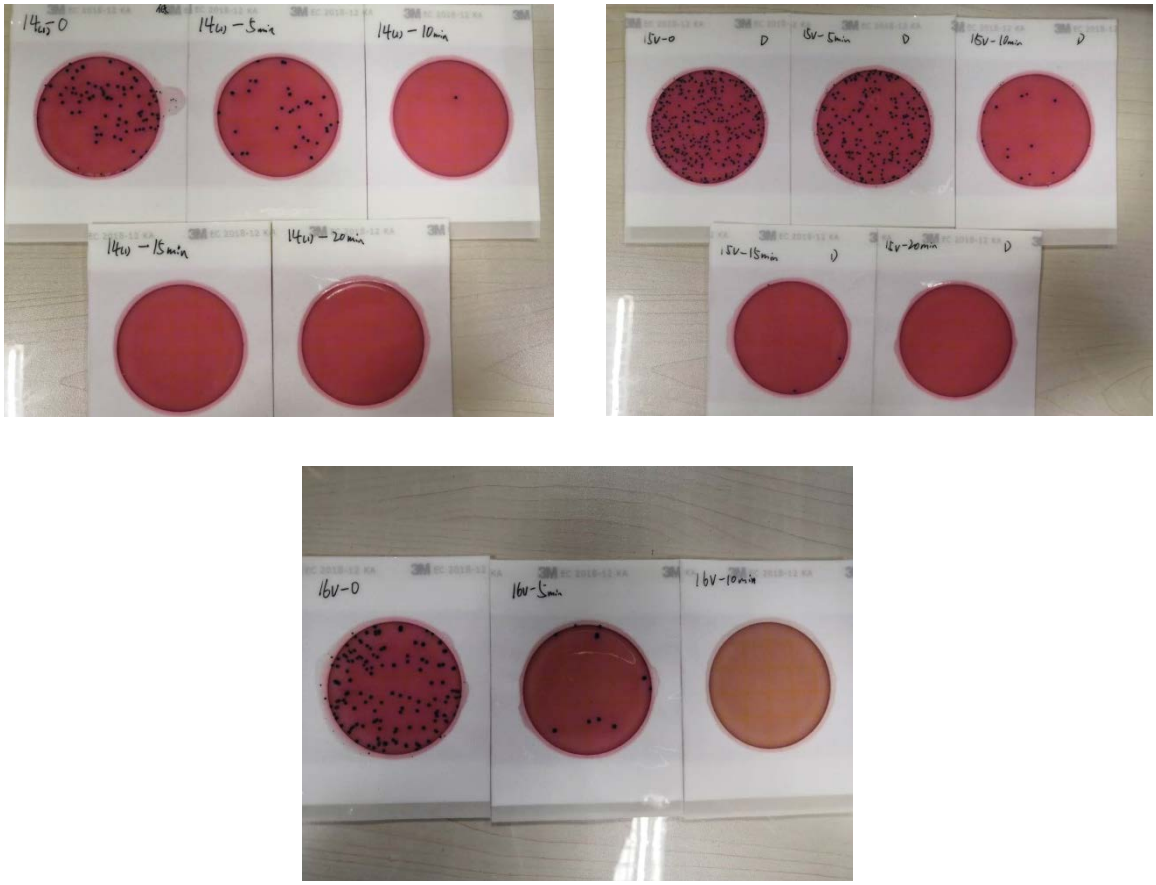

Fig. S3. Images of the cultivated *E. coli* show the concentrations of the bacteria before and after the DEP treatment, corresponding to the data shown in ESI Table S2. From top to bottom, the applied voltage was 14, 15 and 16 V, respectively.