Disinfection of Escherichia Coli in water using moderate electric and magnetic fields

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ABSTRACT

Bacteria play a key role in both human health and disease. One of the most prevalent harmful bacteria is *E. coli* which is responsible for several illnesses ranging from diarrhea, stomach cramp and fever. In this work we explore the application of moderate electric or magnetic fields for treating distilled and deionized water that is contaminated with *E. coli*. We found that moderate alternating current (AC) electric fields (10 V/cm to 1kV/cm) and moderate static magnetic fields (10 mT to 65 mT) can significantly inactivate *E. coli* by up to 90% or more. This provides the possibilities of developing a low cost and a practical bacteria inactivation technique using electric or magnetic fields.

Key words: bacterial inactivation, drinking water, electromagnetic fields, electroporation, *Escherichia coli*

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1. INTRODUCTION

Microbial contamination of food and water is globally considered to be a major health hazard (WHO, 2015). Waterborne pathogens include *Escherichia coli (E.coli)* bacteria (strain O157:H7) which has an infective dose as low as 10 colony forming units (CFU) (Greig, 2010). There are several techniques used for inactivating bacteria and other microbes from water. These include chemical processes such as chlorine disinfection, physical processes such as filtration, UV light treatment, thermal treatment, and the application of electric and magnetic fields (Collivignarelli *et al*, 2017; Johnstone, 1997). There is growing interest in the area of food science on the use of electric and magnetic fields in the decontamination or preservation of liquid food (Vega-Mercado *et al*, 1997; Barbosa-Canovas et al1999; Barbosa-Canovas *et al* and Zhang, 2001; Oziemblowski and Kopec, 2005) . There is also a similar interest in the field of medicine to use electric and/or magnetic fields to increase the efficacy of antibiotics against pathogens (Shawki and Gaballah, 2015; Kermanshahi and Sailani, 2005).

Previous studies have shown that (Giladi *et al*, 2008; Yadollahpour et al, 2014) there are three main factors that influence the destruction of microbes by electric and/or magnetic fields: (i) physiochemical properties of the media (pH, ionic strength, conductivity, and ambient temperature), (ii) microbial characteristics (microbe type, growth stage, and microbe load), and (iii) properties of the applied electromagnetic (E/M) field (field intensity, frequency of field, and exposure time). For electric fields, another contributing factor is the type of electrodes, whether the electrodes are insulated or not. Low intensity electric fields (less than 10 V/cm) or low magnetic fields (less than 1 mT) inhibit microbial growth and cell division processes through electro-phoresis or magneto-phoresis whereby the cellular ions or charged organelles are forced to flow and align in certain directions (Shawki and Gaballah, 2015; Giladi *et al*, 2008). Moderate electric fields (10 V/cm to 1 kV/cm) or moderate magnetic fields (1mT to 1T) have been found to increase the permeability of the cell membrane (Pillet *et al*, 2016; Trainito, 2015; Puc *et al*, 2004; Gaynor and Bodger, 1995;

Filipic *et al*, 2012; Fojt, 2004). The increased permeabilization affects ion transport into the cells and thus can lead to the loss of viability. When the permeabilization is driven by electric (magnetic) fields, the process is called electroporation (magnetoporation), respectively (Eynard et al, 1988; and Rems and Miklavcic, 2016). Electroporation or magnetoporation becomes irreversible at high electric fields (> tens of kV/cm) or high magnetic fields (> 1T). This process explains significant inactivation of bacteria in high electromagnetic fields. For example, (Johnstone and Bodger, 1997) found that the direct current (DC) electric field (about 30 kV/cm) completely inactivates *E coli* inoculated in deionized water within an exposure time of less 17 ms. AC electric fields at the same intensity reduced the bacteria count by three orders of magnitude from an initial viable bacteria load of $10^5 – 10^6$ cells per cm^3.

This work investigates the effects and limits of moderate AC electric fields and static magnetic fields on the survival of *E. coli* in deionized water. Our objective is to develop a low cost and practical technique for drinking water disinfection. Previous studies on the use of moderate intensity electric fields were performed with the *E. coli* in liquid foods Vega-Mercado *et al*, 1997) and also with focus on the effects of frequency of AC fields (Giladi *et al*, 2008) . The inhibitory effects of static magnetic fields were found in *E. coli* in a urine sample (Mousavian-Roshanzamir, 2017).

2. MATERIALS AND METHODS

For the study *E. coli* strain O157:H7 ATCC 35150 pellets were purchased from the American Type Culture Collection (ATCC) company. Bacteria were cultured in eosin methylene blue agar.To obtain a uniform *E. coli* suspension, 0.15 g of *E. coli* concentrate was dissolved in 250 mL of distilled and deionized water. 100 mL of this solution was poured in a beaker and two carbon electrodes were placed inside it on opposite sides (Fig.1). Another 100 mL of this solution was retained as a control. The temperature of both treated and control samples was maintained at $23 \pm 1^{\circ}$C. To study the inhibition /growth rate of the *E. coli* in the presence of applied electric and/or magnetic fields, the following procedure was carried out.

2.1 Alternating (AC) electric field

The electrodes were connected to an alternating voltage source as shown in Fig. 1. The AC voltage at a frequency of 50 Hz, was increased from 5.0 V to 50.0 V in steps of 5.0 V. For each voltage applied, the *E. coli* suspension was exposed to the electric field for a period of one hour and the *E. coli* colony count was measured. Thereafter the time interval was increased in steps of one hour up to a maximum period of eight hours and the colony count was done for each voltage. The colony count enumeration was performed using the Colisure test at the Eswatini Water Services Corporation laboratory in Mbabane, Eswatini. This is an ISO 17025 accredited laboratory. The influence of the E/M fields we obtained from calculating the change in *E. coli* count that is defined by the following ratio:

$$(N_t - N_c)/N_c \qquad (1)$$

where N_t and N_c represent the number of the *E. coli* colonies in the treated sample and control sample, respectively.

2.2 Static magnetic field

The procedure is similar to one applied in the AC electric field experiment (Section 2.1) but the count rate was measured against static magnetic field strength. The data obtained were: (a) colony count versus magnetic field strength for a fixed time interval (2 h) and (b) colony count versus time of exposure for a fixed magnetic field strength (65 mT).

3. RESULTS AND DISCUSSIONS

Variation of *E. coli* count with applied AC electric field for 2 hours is shown in Fig. 2. For AC electric fields with strength between 11 V/cm to 1.1 kV/cm, the bacteria inactivation of 25% to 100% observed. An extremely high decrease in bacteria count was obtained at the lower electric field intensity of 11 V/cm, though these results also depend on the exposure time. For example, at the electric field intensity of 1 kV/cm, decrease in the bacteria count was at least 50% or more for exposure time of above six and half hours as shown in Fig. 2. The effectiveness of electric field of lower intensities, in our study, can be attributed to the electro-mechanical effect that is dependent on the conductivity of the medium (Shawki and Gaballah, 2015; Giladi et al, 2008). Higher electric field intensities can lead to Joule heating effect (Lee et al, 2012) that can affect the bacteria deactivation mechanism.

Variation of *E. coli* count in a sample exposed to various static magnetic fields for two hours is shown in Fig. 3. Again, our results show that magnetic fields between 10 mT and 65 mT have an inhibitory effect on *E. coli.* The maximum reduction in bacteria count occurred at 60 mT with the count declining by 77%. We found that for exposure time greater than one hour, the bacteria count declined by between 40% and 90% at 65 mT limit as shown in Fig. 3. Our results indicate an insignificant decline in the bacteria count for magnetic fields at 20 mT and 30 mT. However, in a recent study (Mousavian-Roshanzamir *et al* 2017) found that a static magnetic field (20 mT) produced a significant decline in survival rate of *E. coli* bacteria in urine samples. This conflicting evidence might be a result of the different physiochemical properties of the media .

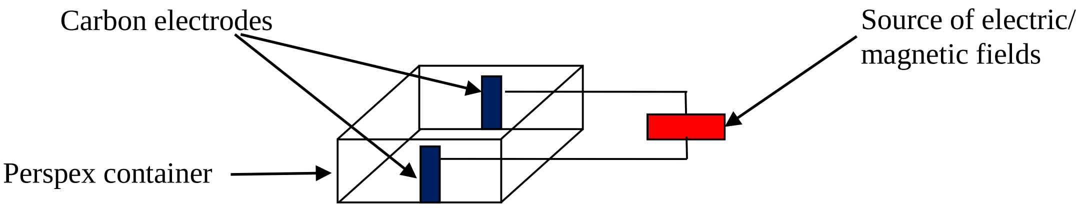

Fig. 1. Schematic diagram of the experimental setup.

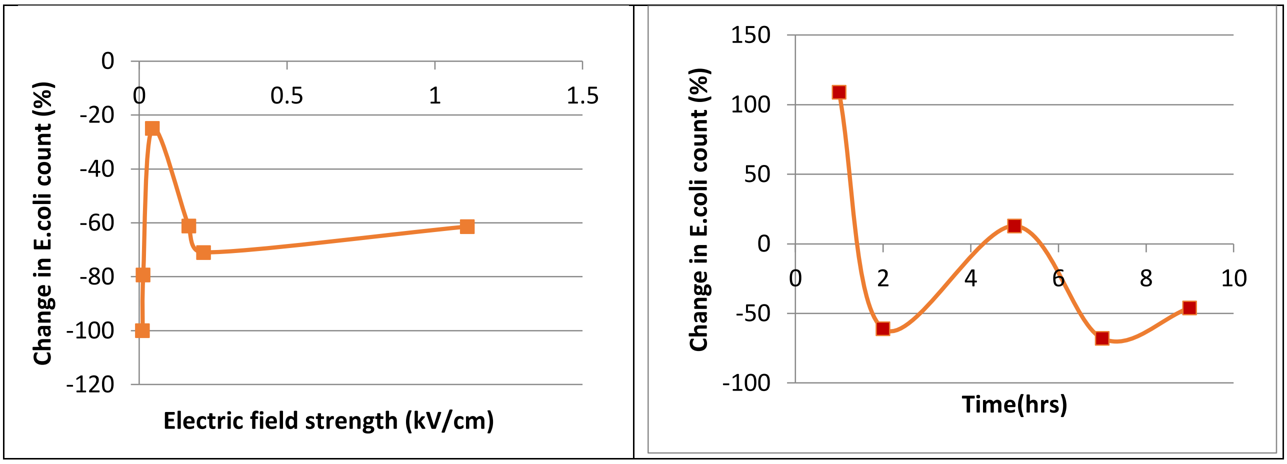

Figure 2: The change in *E. coli* bacteria count (Vs.) exposed AC electric field for 2 hours (left panel). The electric field applied ranged from 0.011 to 1.1 kV/cm. Changes in *E. coli* bacteria count in a sample exposed in AC electric field (1 kV/cm) for different exposure periods (right panel).

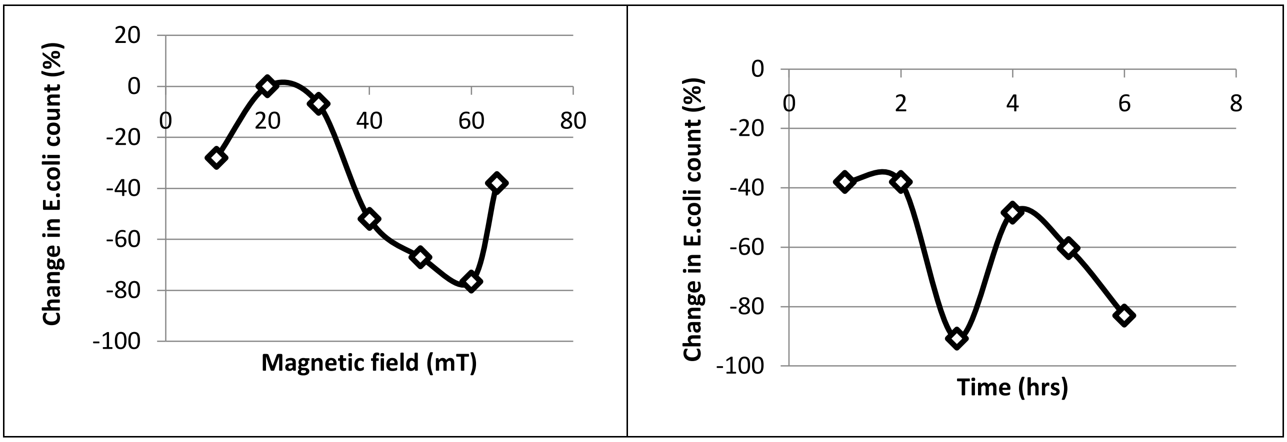

Figure 3: The change in *E. coli* bacteria count exposed to static magnetic fields for 2 hours (left panel). Changes in *E. coli* bacteria count in a sample exposed to a static magnetic field (65 mT) for different exposure periods (right panel).

4. CONCLUSIONS

In summary, the results obtained in this study show that exposing distilled and deionized water contaminated with *E. coli bacteria* to moderate AC electric or moderate static magnetic fields leads to the decline in bacteria count. While previous studies have shown that a high intensity electric field may be more effective as a decontamination of liquid foods, moderate fields are more practical for developing domestic disinfection devices.

It is suggested that more elaborate studies, both on the experimental and theoretical aspects of the subject are needed, placing emphasis on the importance of an ion-free environment for the microorganisms.

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6. REFERENCES

Barbosa-Canovas, G.V., Gondora-Neito, M.M. , Pothakamury, U.R. and Swanson, B.G. (1999). *Preservation of foods with pulsed electric fields.* Academic Press, Washington, D.C.

Barbosa-Canovas, G.V. and Zhang, Q.H. (2001). *Pulsed electric fields in food processing: fundamental aspects and applications.* Technomic Publishing Co., Washington, D.C.

Collivignarelli, M.C., Abba A., Ilaria Benigna ,I., Sorlini, S., and Torretta, V. (2017). Overview of the Main Disinfection Processes for Wastewater and Drinking Water Treatment Plants. Sustainability10, 86.

Eynard, N., Rodriguez, F., Trotard, J., and Teissie, J. (1988). Electrooptics studies of Escherichia coli, electropulsation: Orientation, permeabilization, and gene transfer. Bio. Phys. J. 75, 2587-2596.

Filipic, J., Kraigher, B., Tepus, B., Kokol, V., and Mandic-Mulec, I., (2012). Effects of low-density magnetic fields on the growth and activities of wastewater bacteria Escherichia coli and Pseudomonas putida. *J.* Bioresource Technology 120(4), 225-232.

Fojt, L., Strasak, L., Vetterl, V., and Smarda, J. (2004). Comparison of the low-frequency magnetic field effects on bacteria *Escherichia coli*, *Leclercia adecarboxylata* and *Staphylococcus aureus*. Bioelectrochemistry 63**,** 337-341.

Gaynor, P.T., and Bodger, P.S. (1995). Ballon model of biological cell electropermeabilisation in relation to the radius dependence of membrane dielectric breakdown. IEE Proc. Sci. Meas. Technol. 142(4) 277-282.

Giladi, M., Porat, Y., Blatt, A., Wasserman, Y., Kirson, E.D., Dekel, E., and Palti, Y. (2008). Microbial growth inhibition by alternating electric fields. Antimicrobial Agent and Chemotherapy 52(10), 3517-3522.

Greig, J.D., Todd, E.C.D., Bartleson, C., and Michaels, B. (2010). Infective Doses and Pathogen Carriage. Presentation at the Food Safety Education Conference, Atlanta, USA. *URL:* https://www.fsis.usda.gov/PDF/Atlanta2010/Slides_FSEC_JGreig_Doses.pdf (Accessed 20 March 2019)

Johnstone, P.T. and Bodger, P.S. (1997). High voltage disinfection of liquids. IPENZ Transactions 24(1), 30-35.

Kermanshahi, R.K. and Sailani, M.R. (2005). Effect of static electric field treatment on multiple antibiotic-resistant pathogenic strains of *Escherichia coli* and *Staphylococcus aureus*. Journal of Microbiology Immunology and Infection 38, 394-398.

Lee, S.-Y., Sagong, H.-G., Ryu S., and Kang D.-H. (2012). Effect of continuous ohmic heating to inactivate *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria* monocytogenes in orange juice and tomato juice. J. Appl. Microbiology 112, 723-731.

Mousavian-Roshanzamir, S. and Makhdoumi-Kakhki, A. (2017). The inhibitory effects of static magnetic fields on *Escherichia coli* from two different source at short exposure time. Reports of Biochemistry and Molecular Biology 5(2), 112-116.

Oziemblowski, M. and Kopec, W. (2005). Pulsed electric as an unconditional method of food preservation. Pol. J. Food Nutr. Sci. 15, 31-35.

Pillet, F., Formosa-Dague, C., Baaziz, H. Dague, E.,and Rols, M.-P. (2016). Cell wall as a target for bacteria inactivation by pulsed electric fields. Sci. Rep. 6, 19778.

Puc, M., Corovic, S., Flisar, K., Petkovsek, M., Nastran, J., and Miklavcici, D. (2004). Techniques of signal generation required for electropermeabilization: Survey of electropermeabilization devices. Bioelectrochemistry 65, 113-124.

Rems, L. and Miklavcic, D. (2016).Tutorial: Electroporation of cells in complex materials and tissue. J. Appl. Phys. 106, 024114.

Shawki, M.M. and Gaballah A. (2015). The effect of low AC electric field on bacteria cell death. Romanian J. Biophys. 25(2), 163-172.

Trainito, C. (2015). Study of cell membrane permeabilization induced by pulsed electric field – electric field – electrical modeling and characterization on biochip. PhD Thesis, Universite Paris-Saclay.

Vega-Mercado, H., Martin-Belloso, O., Quin B.L., Cahng, F.J., Gondora-Neito, M.M., Barbosa-Canovas, G.V. and Swanson, B.G. (1997). Non-thermal food preservation: pulsed electric fields. Trends Food Sci. Tech. 8, 151-157.

World Health Organization (2015). WHO estimates of the global burden of foodborne diseases. URL:http://www.who.int/foodsafety/publications/foodborne_disease/fergreport/en/ (Accessed 20 March 2019).

Yadollahpour, A., Jalilifar, M., and Rashidi, S. (2014). Antimicrobial effects of electromagnetic fields: A review of current techniques and mechanism of action. J. Pure Appl. Microbio. 8(5), 4031-4043.