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Microbial inactivation utilizing impulse waveform

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ABSTRACT

Inactivation of *Escherichia coli* has been successfully performed by the utilization of impulse voltage waveform. The process involves in microbial inactivation is pulsed electric field (PEF) treatment. PEF utilizes short-duration high-voltage pulses for microbial inactivation by the process of electroporation. Inactivation rate was measured against peak electric field, number of applied pulses, and energy input to unit volume of liquid. Two different electrode configuration spherical–spherical and parallel-plate electrode systems were used. The results indicate that parallel-plate electrode system results in better inactivation rate than spherical electrode system. With applied electric field of 24 kV/cm and 20 pulses, six log reductions were achieved in parallel-plate electrode system indicating that inactivation rate is strongly dependent on electrode configuration, peak electric field, and applied pulses.

Keywords: Inactivation rate; Pulsed electric field; Impulse waveform; *Escherichia coli*; Drinking water treatment

1. Introduction

Water is an essential constituent of all living organisms. Due to intensive industrial application, almost two-third of total population is deprived of pure water. Number of conventional treatment methods is being used for water disinfection including chemical, physical, and biological methods. But all of these methods are either expensive, inefficient or produce toxic by-products. It has been reported that there is a dire need of an energy efficient, cost-effective and environment friendly disinfection process. It has also been reported that one of the energy efficient and cost-effective disinfection process is the application of high-voltage pulses to the contaminated water [1].

In the past few years, pulsed electric field (PEF) has attracted more and more attention. Microbial disinfection in liquid is of particular importance in food industry, pharmaceutical research, public health, and water purification [2]. One of the earliest applications of electricity in food industry was electric pasteurization of milk. The passage of electric current through milk generates heat which is responsible for bacterial death [3]. As PEF is nonthermal treatment method, the food will retain its physical and nutritional characteristics [4]. Mizuno and Hori [5] investigated the pulsed high-voltage experiments to the liquid for the destruction of *Saccharomyces cerevisiae* and *Bacillus natto* using different electrode configurations and indicated that PEF can be used as a low-temperature sterilization process. Jayaram et al. [6] expressed that survivability of *Lactobacillus brevis* reduces

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exponentially with increase in the treatment time, electric field intensity and temperature by the application of high-voltage DC pulses. Qin et al. [2] illustrated the PEF treatment and reported that inactivation of microorganisms by the application of PEF depends on the waveforms of applied pulses. Sale and Hamilton [9–11] reported the application of rectangular DC pulses up to 25 kV/cm and investigated that microbial inactivation was due to electric field intensity and not because of temperature increase and electrolysis product. Various species of microorganism differed in sensitivity to electric field [2]. Pothakamury et al. [13] investigated the effects of both Gram-positive and Gram-negative bacteria when subjected to high-electric field pulses generated by electroporator. The different structure of the cell walls of Gram-positive and Gram-negative bacteria might interfere in the mechanism of permeabilization [14]. Membrane permeabilization involved in the bacterial inactivation by PEF depends on the treatment media pH and bacterial species (Gram-positive and Gram-negative bacteria). It has been reported that *Lactobacillus plantarum* (Gram-positive bacteria) is less sensitive to PEF treatment than *Escherichia coli* (Gram-negative bacteria) when lower number of pulses applied [15].

1.1. Electroporation

When any external field is applied across the biological cell, charges are accumulated on both sides of membranes of cell, which results in the formation of a dipole. Consider the classic dielectric model of spherical biological cell exposed to DC electric field proposed by Schwan and Grosse [16,17] shown in Fig. 1. According to that model, a biological cell is described by three regions a nonconducting dielectric membrane, conducting cytoplasm and conducting extracellular medium in which cell is enclosed.

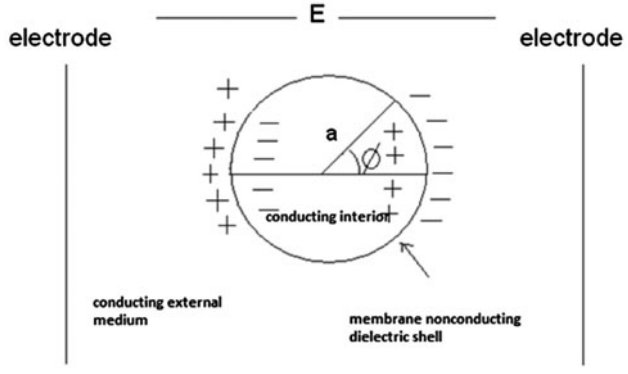

Fig. 1. Electrical equivalent model of biological cell exposed to uniform DC [18].

The membrane normally acts as a capacitor with a dielectric (membrane) between two conductive medium (extracellular medium and cytoplasm). The exposure of PEF to living cells causes dielectric breakdown of membranes when voltage drop across it exceeds 0.5–1 V. It causes the membranes of cell to lose its function due to formation of pores. The process of pore formation in biomembranes by the application of electric field is known as electroporation [2,6]. The critical parameters applied electric field strength, duration of applied voltage (application time) and number of pulses applied, leads to reversible or irreversible breakdown of cell membrane [8]. Electroporation induces a transmembrane voltage "V_m" in the membranes of cell.

$$V_m = f\,Ea\cos\varphi \tag{1}$$

where "f" is the form factor ($f = 1.5$ for spherical cell and $f = 1$ for rod-shaped cells), "E" is the magnitude of applied electric field (V/cm), "a" is cell radius (cm), "$\varnothing$" is the angle from a point on the cell surface to the axis which is parallel to the applied electric field and passes through the cell origin.

2. Methods

2.1. Microbiology

E. coli was used in this experiment which was procured from Life sciences Centre of COMSATS Institute of Information Technology, Abbottabad, Pakistan. *E. coli* has a rod-shaped, with 1.2–5.2 μm long and 0.6–0.7 μm in diameter. For cell destruction, *E. coli* is cultured using standard nutrient broth and incubated at 37°C for 24 h until it reaches the early stationary growth phase [7]. The cells were then centrifuged at 4,000 rpm for 15 min. In order to obtain a high-voltage gradient, the cells were suspended in sterilized deionized water. The concentration of cell before and after treatment was determined by cultivation method. The sample was first serially diluted and implanted on the surface of NA agar culture medium in Petri dish [7]. The sample was than incubated for 24 h, and colonies were counted. The dilutions were done in such a way as to give 30–300 colonies per plate [12]. The initial concentration of cell was 18×10^3–18×10^5 CFU/ml.

Inactivation of microorganisms is determined by survivability rate given by

$$s = \frac{N}{N_0} \tag{2}$$

where N_0 and N are number of CFU/ml before and after treatment.

2.2. Impulse generator circuit

Impulse voltage waveform is generally described by peak value, wave rise time (time required to reach 90% of its peak value) and wave fall time (time required for impulse wave to decay 50% of its peak value. The schematic diagram of impulse generator is shown in Fig. 2. A capacitor "C_1" is charged through high-voltage dc. By closing switch "S", it is discharged into wave shaping network (R_1, R_2 and C_2). The discharging voltage "v_0 (t)" gives rise to double exponential wave shape.

As generator capacitor C_1 and load capacitor C_2 are fixed and their values depend upon the design of generator and load. In order to obtain the desired wave rise and fall time, the values of R_1 and R_2 were adjusted. Wave rise time "t_1" is defined a follows:

$$t_1 = 3R_1 \frac{c_1 c_2}{c_1 + c_2} \tag{3}$$

where $R_1 = 350\,\Omega$ is the wave front, $R_2 = 2{,}400\,\Omega$ is wave tail resistor. $C_1 = 25$ nF is the impulse capacitor and $C_2 = 1.2$ nF is the load capacitor. Wave fall time "t_2" is defined as follows:

$$t_2 = 0.7(R_1 + R_2)(C_1 + C_2) \tag{4}$$

By substituting values, we get $t_1 = 1.2\,\mu$s $t_2 = 50\,\mu$s determines the applied field duration.

2.3. Electrode system

The design of electrode system is an important aspect for PEF to be used as a nonthermal treatment process. The spherical electrode system used in this experiment is shown in Fig. 3(a). The separation between two electrodes was 2.5 mm. The electrodes were contained in a chamber made up of Perspex with dimension $14 \times 10 \times 11$ cm, and the volume was 350 cm^3. The parallel-plate electrodes shown in Fig. 3 (b) were contained in plexiglass chamber. The length of electrode was 25 cm, and volume was 50 cm^3. The separation between two electrodes was 1 cm.

2.4. Necessary conditions for inactivation

If applied electric field across biological cell remains after the initial breakdown of membranes of cell, it causes the membrane to be permanently permeabilized, pores created are sufficiently large in size which causes complete destruction of cell and it is termed as irreversible electroporation. Irreversible electroporation results in complete cell inactivation. Necessary conditions for successful inactivation are the following;

Applied electric field strength E_p should be greater than critical electric field strength E_c i.e.

$$E_c = \frac{V_c}{fa} = 22.2\ kv/cm \tag{5}$$

V_c = critical transmembrane potential and its value is 1 V, f = form factor and its value is 1 and a = radius of cell and its value is 0.3 μm.

Applied field duration should be greater than charging time constant of membrane τ i.e.

$$\tau = [\rho_i + 0.5\rho_e]C_m a = 420\ \text{ns} \tag{6}$$

ρ_i = resistivity of intracellular medium its value is $10^{-4}\,\Omega$ cm, ρ_e = Resistivity of extracellular medium in which cell is suspended its value is $0.04 \times 10^6\,\Omega$ cm, C_m = capacitance of cell membrane and its value is 0.7 μF/cm^2.

In order to avoid electrolysis problem and temperature rise in the treatment chamber, electric field must be applied in short duration pulses.

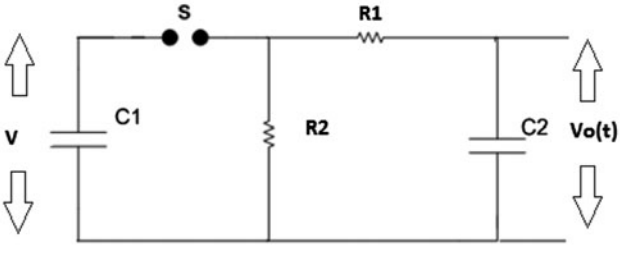

Fig. 2. Schematic diagram of impulse generator.

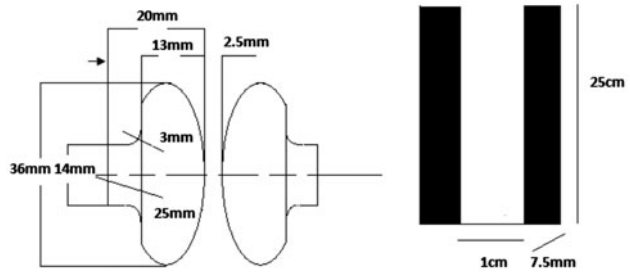

Fig. 3. Electrode system (a) spherical electrodes and (b) parallel-plate electrodes.

3. Results and discussion

3.1. Impulse waveform and energy input

A lightning impulse voltage with rise time of 1.2 μs and fall time of 50 μs is applied to the test cell. For PEF to be used as a nonthermal sterilization process, energy consumption is one of the most significant points to be considered. Energy input to liquid/unit volume by number of pulses applied is;

$$W_i = 1/2\frac{V_0^2 nC}{vol} \quad (\mathrm{J/cm^3}) \tag{7}$$

3.2. Inactivation rate

3.2.1. Spherical electrode system

Fig. 3 shows the survivability rate of *E. coli* dispersed in deionized water against pulses applied. The spherical electrode system was used. The peak voltage was 6, 8, 12 and 16 kV Results indicate that survivability rate is an exponential decaying function of applied number of pulses. At $V_0 = 6$, 8 and 12 kV, the decrease in survivability rate was quite slow and complete cell destruction could not be achieved even by the application of 120 applied pulses. The total energy input W_i was 0.03 cal/cm^3 at $V_0 = 6$ kV, 0.06 cal/cm^3 at $V_o = 8$ kV, 0.1476 cal/cm^3 at $V_o = 12$ kV. At $V_o = 16$ kV, survivability rate was reduced to 10^{-1} by the application of 50 pulses and 2.5 ms treatment time. The total energy input W_i was 0.26 cal/cm^3 (Fig. 4).

In case of spherical electrode system, electric field was higher in a region near to electrode while in other regions electric field was low. Due to nonuniformity of field, all cells were not equally exposed to electric field, so cell destruction performance was not so effective. To improve the overall performance of cell destruction, magnetic stirrer was used during PEF application. The liquid was continuously agitated with the help of a magnetic stirrer. So that electric field is equally distributed throughout the region. By the continuous movement of cell in the vicinity of electrode, survivability rate reduces to 10^{-3}. The effect of agitation on survivability of *E. coli* is shown in Fig. 5.

3.2.2. Parallel-plate electrode system

The survivability rate was measured using parallel-plate electrode system. The peak voltage was 23 and 24 kV. Fig. 6 shows the survivability of *E. coli* against number of applied pulses.

For any applied electric field less than critical electric field, no inactivation was achieved. In case of $E_p = 20$ kV/cm, survivability rate was the maximum. As E_p increases beyond E_c, survivability rate was reduced. Results obtained from above graphs indicate that by using parallel-plate electrode configuration, the inactivation rate was high and at $E_p = 20$ kV/cm survivability rate reduces to 10^{-6} by the application of lower number of pulses applied. No colonies were detected at $Vo = 24$ kV and 20 applied pulses. Thus, successful inactivation of *E. coli* was achieved with little energy consumption. The total energy input W_i

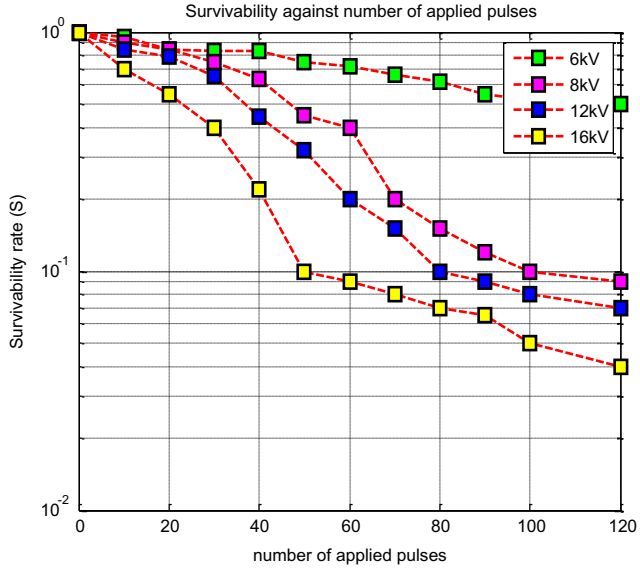

Fig. 4. Survivability rate of *E. coli* against number of applied pulses using spherical-electrode system at different voltage magnitude.

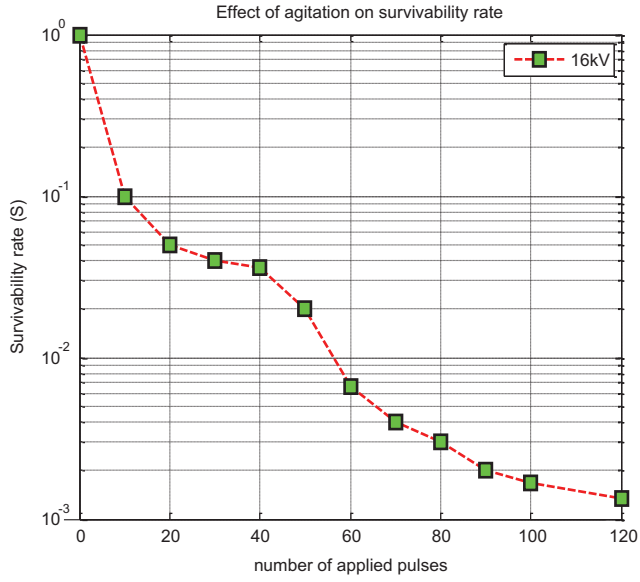

Fig. 5. Effect of agitation on survivability rate of *E. coli*.

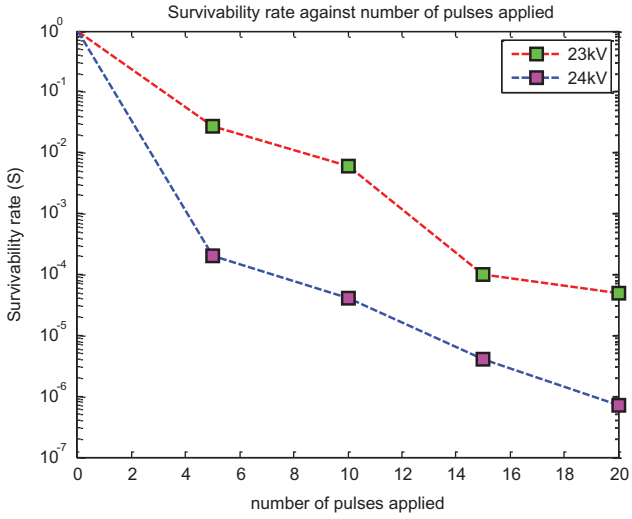

Fig. 6. Survivability rate of *E. coli* against number of applied pulses using parallel-plate electrode system at different voltage magnitude.

was 0.6912 cal/cm^3 with 1 ms treatment time. Comparing the inactivation rate of both electrode configurations it is concluded that parallel-plate electrode results in better inactivation rate.

3.3. Comparison with existing PEF techniques

Table 1 shows the comparison of PEF utilizing impulse waveform with existing PEF techniques. Comparison shows that non-thermal PEF treatment method by the application of high-voltage impulse waveform is proved to be environment friendly, energy efficient, and cost-effective treatment method comparing with the existing PEF techniques by considering the joint effects of peak applied voltage, number of applied pulses and applied field duration on the inactivation of *E. coli*.

Electrical sterilization of biological cells has the advantage of conventional, chemical or thermal sterilization and has been studied by many researchers. In the past few years, different researchers focused on the PEF effects on cell inactivation using different voltage waveform, applied electric field intensity, number of applied pulses and time duration. Qin et al. [2] applied DC electric field on flowing volume of water and obtained 10^{-3} survivability rate of *S. marcescens* with 17 ms application time. However, DC field poses electrolysis problem and the injection of metal ions in water could thus occur in DC. Mizuno and Hori [5] investigated the effects of pulsed high-voltage experiments on *S. cerevisiae* and *Basillus natto* and obtained 10^{-6} survivability rate by using wire-cylinder electrode and 250 numbers of applied pulses. Qin et al. [2] obtained the 10^{-6} survivability rate of *Bacillus subtilis* by applying 16 kV/cm bipolar electric field intensity and number of applied pulses was 100. Applied energy was 120 J for one bipolar pulse. Matsumoto et al. [7] proposed converged electric field (CEF) for the inactivation of *E. coli* and *S. cerevisiae* and obtained 10^{-6} survivability rate by the application of 40 kV/cm electric field intensity and 20 numbers of applied pulses. However, energy input was 25–30 cal/cm^3. However, the current study involved the application of the process in drinking water treatment. Two different electrode configuration spherical-spherical and parallel-plate electrode systems were used. The results indicate that using parallel-plate electrode system, with applied electric field of 24 kV/cm and 20 pulses, six log reductions were achieved with low energy input and 1msec treatment time. Comparing current results with the previous researches [19–21], it is concluded that PEF treatment utilizing impulse waveform is proved to be more energy efficient and complete cell destruction is achieved with lower pulses applied and minimum treatment time indicating that inactivation rate is strongly dependent on peak electric field and applied pulses. Thus, the process has promising perspectives for its application in the drinking water treatment.

Table 1
Comparison with existing techniques

PEF technique (applied waveform)	Applied electric field (kV/cm)	Survivability rate	Inactivated microorganisms
Impulse waveform (current research)	24	10^{-6}	*Escherichia coli*
DC electric field [1]	30	10^{-3}	*Serratia marcescens*
Bipolar waveform [2]	16	10^{-6}	*Bacillus subtilis*
Exponential decay [5]	40	10^{-6}	*Saccharomyces cerevisiae*
CEF [7]	40	10^{-6}	*Escherichia coli*, *Saccharomyces cerevisiae*

4. Conclusion

It can be concluded that cell inactivation has been performed effectively by the application of high voltage impulse waveform. Application of impulse

waveform to the treatment cell generates intense electric field across the biological cell. Due to intense electric field, pressure arises on the wall of cell membrane, which results in mechanical disruption as well as dielectric breakdown of membrane. While other waveforms utilized in PEF treatment do not generate intense electric field and only dielectric breakdown of the membrane takes place. Thus, impulse waveform results in better inactivation rate with minimum energy consumption and treatment time than other waveforms utilized in PEF treatment.

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