High Voltage Pulsed Electric Field Application Using Titanium Electrodes for Bacterial Inactivation in Unpurified Water

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Pulsed Electric Field (PEF) treatment is considered as nonthermal due to application of ultra short high voltage pulses in liquid foods to extend their shelf life. In today’s world, water decontamination becomes extremely necessary to safeguard people from health ailments. The objective of this work focuses on inactivation of naturally prevailing _Escherichia coli_ and _Fecal coliform_ bacteria in environmental water using titanium electrodes. In this study, the PEF treatment chamber was designed to be used for both static and continuous modes of treatment. Bipolar square wave pulses having 1 μs pulse width at a rise time of 160 ns and pulse repetition frequency between 48 to 50 Hz were used in this research. From the results, it was observed that titanium effectively inactivated both the microorganisms at a minimum treatment time of 60 seconds at 33.9°C while conventional stainless steel required 120 seconds at a temperature of 40.1°C under the same experimental conditions. Also, the relationship between treatment time and temperature remained linear despite the change in electric field. Results confirmed that (i) Titanium is more suitable in PEF for water decontamination due to its high reactivity than stainless steel (ii) Using titanium, complete ABSENCE of the two microorganisms could be possible in water at a nominal field strength of 24 kV/cm with much less temperature requirement.

**Keywords:** Pulsed Electric Field, Titanium Electrodes, Water Decontamination, _Escherichia coli_ and _Coliform._

1. Introduction

Pulsed Electric Field treatment (PEF) has a greater potential for inactivating viable pathogens present in liquid food products through electroporation [1–4]. The electric field can be applied in the form of ultra short high voltage pulses (20 kV/cm to 80 kV/cm) to liquid food present in PEF treatment chamber accompanying two electrodes. In previous PEF studies, stainless steel was generally recommended as the electrode material [1, 5–9]. Though other metals have also been used, the use of titanium is still limited in PEF and needs further growth in applications. So far, PEF has given successful inactivation rates in various fruit juices, but it’s role on water decontamination is still not fully developed to make the technology a complete one. Water contamination is a major concern today, as the bacteria in water pose a greater health risk when consumed. In this work, water was chosen as the testing liquid because it is one of the basic needs for everyday consumption. If water is not properly treated and consumed, it can lead to stomach related diseases. Hence this work focusses on two major factors. (i) Electrode Material – In most of the PEF observations, stainless steel electrodes were used as the state of art material which has given effective inactivation rates. But when the corrosion properties of stainless steel were analyzed, the material reported more metal ion release than titanium [10,11]. When titanium is considered, it is a more durable metal which has been used extensively in surgical implants and in cooking utensils due to its nontoxic properties. It is less dense and has high strength than stainless steel. While stainless steel is basically an alloy made up of a mixture of chromium, iron and sometimes other metals to enhance its corrosion resistant property, titanium’s characteristics are naturally found within it. Under fluctuating changes in temperature, titanium is a better choice than stainless steel.
because it is highly resistant to fatigue. Titanium is also more reactive than stainless steel because it reacts readily with oxygen and forms stable and protective oxide layer on the metal surface. Due to this oxide layer, it has excellent corrosion resistant property. Even when oxide layer gets damaged, it will heal itself instantaneously if traces of oxygen or water is present in the environment [12]. The resistivity of titanium ranges from $4.2 \times 10^{-7}$ to $5.2 \times 10^{-7}$ ohm-m [13] whereas for stainless steel it is $7.4 \times 10^{-7}$ ohm-m [13].

Previous studies used either uncoated or platinum coated titanium electrodes for electrolysis treatments including water ionizers, waste water electrolysis treatments, electrochemical and electrocoagulation treatments due to its excellent electrochemical stability [14-16]. These treatments rely on application of continuous electric current passing through the water for disinfection which may require high temperature and treatment time and also may result in higher concentration of metal ion release into the liquid medium but in PEF, electric current plays only a trivial role and the microbial inactivation depends on high voltage pulse application in microseconds. Hence, the methodology adopted and mode of application for all the above techniques are different when compared with PEF, other than the use of titanium electrodes as one of their electrode materials. Recent research highlighted the potential of titanium on higher retention of ascorbic acid than stainless steel under ohmic heating method [17]. While very few studies used titanium for PEF applications in liquid foods [18], neither of previous work has focused on reaction of titanium in water under PEF treatment. Hence, under this study titanium was chosen as the electrode material. (ii) Water decontamination – People in rural areas drink unpurified water from several sources. Hence it was proposed to decontaminate unpurified water using titanium electrodes under PEF treatment, targeting the most naturally surviving Escherichia coli (E.coli) and Coliform bacterial loads. Many water treatment methods are available today which are considered as effective including UV systems, Ozone water purification, chlorination and especially reverse osmosis (RO) water purifiers. These water treatment methods have their own disadvantages. For instance, while UV and ozone systems have the potential to induce carcinogenic effects, chlorination involves addition of chemicals. Though reverse osmosis water treatment is considered as a complete technology for water purification, it has the drawback of demineralizing the water and wasting 3-4 liters to purify one liter of water [19]. The wasted water also becomes non consumable. But PEF can effectively decontaminate water with the following advantages (i) No thermal effect (ii) No addition of chemicals (iii) No water wastage (iv) No hazardous effects on health (v) No demineralization.

2. Materials and Methods

2.1 High Voltage Pulse Generator

A high voltage pulse generator is used for delivering high electric field pulses to liquid food placed between two electrodes in PEF treatment chamber. Fig. 1 shows the circuit of high voltage pulse generator consisting of a 10 stage Pulse forming network (PFN). In this research, the pulse forming network is designed to deliver bipolar square waveforms having positive and negative polarities, where each polarity has a pulse width of around 1 $\mu$ S. Basically, the pulse forming network comprises of high energy storage components such as capacitors, inductors, and transmission lines which can be charged by a high voltage DC power source, and then rapidly discharged into load (PEF treatment chamber).

In other words, the voltage from the supply is stepped up and rectified into high DC voltage and then is given in the form of pulses to the load through pulse forming network (PFN). A spark gap switch is employed between

![Fig. 1 Circuit of square wave pulse generator.](image-url)
the PFN and load for switching high voltage pulsed power applications. A resistor $R_s$ of 100 MΩ is connected across the spark gap switch to protect the device during breakdown or under dynamic high voltage conditions. Previous studies reported the efficiency of using square wave pulses for bacterial inactivation when compared with other waveshapes [5,20,21]. Bipolar pulses were chosen in this research due to better bacterial inactivation, reduction of electrolysis with undesirable temperature increase in liquid food [5,20,21].

Since pulse forming networks represent approximation for a transmission line, they must be charged up to twice the desired output pulse voltage since half of the voltage will be dropped across the PFN impedance and the remainder across the load impedance. Hence in this work, the voltage delivered to the load $V_l$ will be half that of the voltage $V_0$ charged through the capacitor bank from $C_1$ to $C_{10}$. Here, $V_0$ is the no load charging voltage and $V_l$ is the load voltage or the voltage across the electrodes. The value of capacitance for each charging capacitor ($C_1 - C_{10}$) is 1000 pF, resistance of charging resistor $R_c$ is 1 kΩ, Inductance value for each Inductor ($L_1 - L_{10}$) is 2.5 μH with a voltage rating of up to 100 kV are used in the PFN respectively. A resistor $R_L$ with a resistance of 50 Ω is connected across the capacitor bank before the pulsed energy getting discharged to the load for impedance matching purposes. If the characteristic impedance of the PFN is matched to that of the load, the energy will be dissipated to the load without any further voltage drop or reflection, where the voltage across the load will be one half the charged voltage of the PFN capacitors as previously stated. Here, the charging voltage across the electrodes play a major role in generating the required electric field for bacterial inactivation.

The current will be less significant in this study and the value will be negligible in few milliamps. For instance, in this study the resistance across two electrodes in water sample is measured as 9.5 MΩ. According to ohms law, when the charging voltage across the electrodes is 4 kV, the corresponding current drawn by them will be only 0.5 mA. Then the generated electric field is calculated theoretically as [22]

$$E = \frac{V}{d}$$

Where $V$ is the charging voltage across electrodes and $d$ is the distance gap between them. Thus, Inactivation of microbes can be successfully obtained using ultrashort high voltage pulse application under PEF treatment. We can observe the cursor measurement representing the total pulsedwidth of a bipolar square waveshape having positive and negative polarities, each having a pulsedwidth of around 1 μS applied across the electrodes in water as shown in Fig. 2.

The specifications for the pulsed input are shown in Table 1

### Table 1 Specifications for pulse profile

| Pulse Profile            | Specification |
|--------------------------|---------------|
| Wave Shape               | Square        |
| Polarity                 | Bipolar       |
| Pulsedwidth for each polarity | ~1 μS        |
| Pulse Repetition Frequency | (48 – 50) Hz  |

2.2 Instrumentation

A 100 MΩ ($R_{m1}$) 1000X Tektronix voltage compensated probe is connected across the treatment chamber and Tektronix TDS 2022 oscilloscope (bandwidth of 200 MHz and peak sample rate of 2 GS/s) for high voltage measurements. Temperature of the water was monitored after the treatment using Instrumentics digital food thermometer which can measure food temperature from –50°C to +300°C.

2.3 PEF Treatment Chamber

Parallel plate electrodes were used in this research as shown in Fig. 3, which can provide uniform electric field distribution in the treatment region. Previous studies used round parallel plate stainless steel electrodes due to the advantage of having simple geometrical dimensions and uniformly treated liquid in the treatment area [5]. In this research, the circular edges of electrodes were smoothened to avoid possible field fringing on those...
edges. The PEF chamber is made up of autoclavable acrylic material (Perspex) with inlet or filling port and liquid release valve. The novelty lies in the chamber design, which can be used for both static and continuous modes of PEF treatment and also have a screw arrangement so that the distance gap $d$ between two electrodes can be varied. The volume of PEF treatment chamber accompanies 120 ml of liquid to be tested. The diameter and thickness of the electrodes used were 40 mm and 5 mm respectively. The inner cylindrical volume has a diameter and height of 70 mm and 50 mm respectively.

### 2.4 Sterilization of Experimental Equipment
Before performing the experiment, the chamber was autoclaved, cleaned with warm soapy water followed by isopropyl alcohol and sterile water. The immersing section of food thermometer was disinfected with isopropyl alcohol before measurement.

### 2.5 Microbial Parameters
The microbial parameters chosen for inactivation were *E. coli* and *Fecal Coliform* bacteria, which are naturally present and predominantly surviving microorganisms in environmental and domestic tap water. The water sample was collected from a remote area in Karnataka from a public tap. The collecting vials were thoroughly sterilized before filling up with untreated water for maintaining accuracy. After confirming the presence of these two microorganisms in tap water, further analysis was initiated. The reference microorganism used for identifying the type of bacteria in water was *E. coli MTCC 433* which is a rod–shaped gram–negative bacterium. In other words, the identification of *Ecoli* was performed by comparing with standard *E coli MTCC 433*. This microorganism can live in human intestines and can cause stomach related illness in humans [23]. *Coliforms* will be usually found in the environment, where feces of man and other warm–blooded animals will be present. The presence of *coli* form bacteria in environmental water may relate to the presence of harmful, disease causing microorganisms. For the microbial analysis, PRESENCE / ABSENCE method was adopted

### 2.6 Water sample analysis
Water sample was analyzed for *E. coli* and *Coliform* bacteria after every PEF application. While bacterial count was emphasized in previous research studies, complete absence of bacteria was primarily required in this research to ensure water quality and safety for everyday consumption.

#### 2.6.1 Membrane Filtration Method
The [Indian standard method IS 15185:2016](https://www.isi.gov.in/) (International standard equivalent ISO 9308-1:2014) was adopted for evaluation and detection of *E. coli* and *coli* form bacteria in water samples after application of high electric field. This method is usually recommended for bacteriological examination of water. Under this method, the sample is passed through a membrane using a filter funnel and vacuum system as shown in Fig. 4a. The presence of microorganisms will be trapped on the membrane surface. This membrane with bacterial concentra-
tion will be placed in a special glass plate equipped with a pad saturated with appropriate growth medium. The passage of nutrients through the filter during incubation period enables the growth of microorganisms on the upper membrane surface as shown in Fig. 4b. The bacteria thus grown can be easily transferred to confirmation media. Membrane filter technique is thus an effective, accepted technique for testing fluid samples for microbiological contamination.

2.6.2 Significance of PRESENCE – ABSENCE (P/A) approach

IS 10500 is the most recommended Indian standard for indicating the limits in bacterial parameters in water. The result will be interpreted as PRESENT irrespective of any number of viable pathogens present in the water and will not be certified as safe for drinking purposes. The result will be interpreted as ABSENT for complete absence of bacteria which is primarily required, satisfying the objective of this research work. Hence according to IS 10500, E. coli and Coliform bacteria should be absent or not be detectable per 100 ml of water for certification as safe for everyday consumption. However, data on initial concentration was required for concise approach. Accordingly, the initial concentration of E.coli and coliform bacteria was found to be around 500 MPN and 700 MPN per 100 ml of untreated water, where MPN represents most probable number of bacterial densities found in 100 ml of water sample.

3. Results and Discussion

3.1 Impact of electric field and treatment time on microbial inactivation

Treatment time is expressed as multiplication of number of pulses by pulse width. Increasing either of the two will result in higher microbial inactivation. In this research, pulse width was made constant throughout the experiment and number of pulses were increased. According to previous studies, by maintaining a constant pulsed width, the energy consumption can be reduced [24]. When the number of pulses increases, the associated treatment time will also increase. Hence, the treatment time was varied from 30 to 360 seconds for titanium electrodes and from 60 to 180 seconds for stainless steel electrodes. Since the research focus was on titanium electrodes, comparative study with stainless steel was made only with observations showing difference in the results. Here, the field was gradually increased to prevent sudden sparking between the two electrodes. The general range of electric field strength used in PEF treatment will be from 20 kV/cm to 80 kV/cm for getting good inactivation results. However, there are some studies which has given inactivation rates at lesser field values [25,26]. Since the novelty lies on the treatment chamber design, it was necessary to determine a suitable field value which should obtain effective inactivation rates using this chamber design. Hence, determining the field value was based on two important factors (i) Should be a nominal value, which should not induce thermal effects (ii) Should have inactivation effect on both E.coli and Coliform bacteria.

For the purpose, initial experimentation was carried at a distance gap of 5 mm, which generated electric field of 9.4 kV/cm at a treatment time of 30 seconds. Results reported that at this distance, the field generated was not sufficient to inactivate both the bacteria and the corresponding treatment time was found to be less effective on these microorganisms. It was also noted that visual effects of discharges indicating the reaction between the electrode and water could not be seen at 30 seconds. This initial observation aided in fixing the suitable field value and a starting treatment time for further applications. Accordingly, the distance gap was adjusted and reduced to 2 mm which generated a field strength of 24 kV/cm. At this field, the corresponding treatment time was set for 60 seconds. While the pulses were continuously applied, a notable reaction of visual discharges was observed at 24 kV/cm when the treatment time approached 60 seconds.

Under these conditions, both the bacteria were completely inactivated i.e., a complete absence indicating zero bacteria at a temperature of 33.9°C using titanium electrodes. The following inactivation results are shown in Table 2.

From the above results, the microbes were inactivated from 60 seconds onwards at a field strength of 24 kV/cm. Hence, this time was taken as a standard of comparison with stainless steel under the same experimental conditions. Using stainless steel, observations were made till 180 seconds as further increase in treatment time resulted in saturation and all the necessary results were obtained within the time course followed. The corresponding results are shown in Table 3.

In the case of stainless steel, while absence of E.coli and presence of Coliform was observed at 60 seconds, the metal took 120 seconds to inactivate both the microorganisms at a temperature of 40.1°C. Hence only half of the treatment time was taken by the titanium electrodes.
3.2 Impact of electric field and treatment time on temperature increase

A complete ABSENCE of bacteria in water was obtained at 60 seconds and 120 seconds using titanium and stainless steel electrodes respectively. After getting the inactivation results, saturation effects started to emerge. Under this condition, the relationship between other parameters such as the treatment time, electric field and temperature was studied. While temperature control is an important parameter to be considered in PEF in fruit juices to retain the food’s quality attributes, it is also significant in the case of water for reduced energy consumption than other heat treatments. Hence optimization of electrical parameters was required to get better inactivation in PEF at less temperature. The electric field and treatment time are the two important input parameters that will influence the temperature increase in liquid food. Under this study, the temperature was monitored for (i) Constant electric field of 24 kV/cm (ii) Sudden reduction of electric field from 24 kV/cm to 20 kV/cm at 180 seconds in the mid of experimentation. Under both circumstances, the temperature increased linearly for increase in treatment time. Though the field was reduced suddenly, the treatment time was still in increasing mode and hence temperature also increased. From the observations, it was understood that increase in treatment time had a primary impact on temperature increase than change in electric field. Thus, reduction in field did not influence the linear relationship between the treatment time and temperature. This study confirmed that the chosen field value was in nominal range. The linearity can be observed in the graphical data for titanium and stainless steel as shown in Fig. 5a and 5b respectively.

Table 2: Inactivation results for Titanium Electrodes

| Electrode Material | Electric Field (kV/cm) | Distance gap between electrodes (mm) | Treatment time (seconds) | (P/A)* per 100 ml of water sample |
|--------------------|------------------------|-------------------------------------|--------------------------|----------------------------------|
| Zero Field (Untreated Water) | 9.4 | 5 | 30 | P | P |
| Titanium           | 24 | 2 | 60 | A | A |
|                     | 24 | 2 | 90 | A | A |
|                     | 24 | 2 | 120 | A | A |
|                     | 24 | 2 | 150 | A | A |
|                     | 24 | 2 | 180 | A | A |
|                     | 24 | 2 | 240 | A | A |
|                     | 24 | 2 | 300 | A | A |
|                     | 24 | 2 | 360 | A | A |

*P/A=Presence/Absence of bacteria per 100 ml of testing water sample

Table 3: Inactivation results for stainless steel electrodes

| Electrode Material | Electric Field (kV/cm) | Distance gap between electrodes (mm) | Treatment time (seconds) | (P/A)* per 100 ml of water sample |
|--------------------|------------------------|-------------------------------------|--------------------------|----------------------------------|
| Zero Field (Untreated Water) | 24 kV/cm | 2 | 60 | A | P |
| Stainless Steel    | 24 kV/cm | 2 | 90 | A | P |
|                     | 24 kV/cm | 2 | 120 | A | A |
|                     | 24 kV/cm | 2 | 150 | A | A |
|                     | 20 kV/cm | 2 | 180 | A | A |

*P/A=Presence/Absence of bacteria per 100 ml of testing water sample
4. Conclusion

This research work has shown positive results on the use of titanium over stainless steel. (i) Titanium has shown to be more effective than stainless steel on inactivation of *E.coli* and *Coliform* bacteria, which was possible at a much lesser time of 60 seconds while stainless steel required 120 seconds for reaction (ii) Complete ABSENCE of bacteria was possible at 33.9°C using titanium which was found to be lower than temperature requirement of 40.1°C in stainless steel under the same experimental conditions. It is also understood that treatment time had a primary impact on temperature increase than electric field. Future research is based on pH analysis, filtering of impurities and inactivation of other bacterial loads in water to make PEF a complete technology for water decontamination. Hence the combination of anticorrosive and highly reactive titanium along with ultra short PEF application provides effective bacterial inactivation on drinking water at a very short treatment time with low temperature requirement.

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