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Inactivation of Microbes using Pulsed Electric Field

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Abstract: The extended preservation of food has been a challenge for mankind throughout the ages due to presence of microbes in every considerable niche of human environment. The present work is based on Pulsed Electric Field treatment, where the liquid foods contaminated with microorganisms are subjected to application of Pulsed voltages in parallel plate static treatment chamber. This study examines the reduction in survival ratio after application of pulsed electric field on four organisms namely Bacillus cereus, Klebseilla pneumonia, Staphylococcus aureus and Pseudomonas aeruginosa when subjected to impulse wave of 1.2/50μs in parallel plate treatment chamber. The treatment voltage was varied in the range 20-80 kV and the number of treatment pulses varied in the range of 25 -100 pulses. The results showed that Staphylococcus aureus was the most sensitive organism with a 7-order reduction followed by Bacillus cereus and Klebseilla pneumonia with a 6-order reduction, when they were exposed to 80KV and 100 pulses. The most resistant organisms were Pseudomonas aeruginosa with only a 4-order reduction.

Keywords: Microbes, Inactivation, Non-Thermal, PEF.

I. INTRODUCTION

The majority of foods harbour several types of microorganisms [1]. Some of them have desirable roles in the food industry, such as in the production of fermented foods, whereas others cause food spoilage and human diseases. Bacteria such as Bacillus cereus, Klebseilla pneumonia, Staphylococcus aureus and Pseudomonas aeruginosa may be present in foods and generate public health problems. Thus, a minimal processing of fresh products to decrease or eliminate bacteria without significantly affecting their nourishing attributes is required. Inactivation depends upon microbial entity like type, concentration, and growth stage of microorganism [2].

The objective of food preservation technologies used by food industry is to control microorganism's growth. These are broadly divided in thermal and non-thermal technique. Thermal processing is very effective technology for microbial inactivation; however excessive heat treatment may cause undesirable effects on foods such as protein denaturation, non-enzymatic browning and loss of vitamins and volatile flavours.

The ever-increasing trend toward nutritionally qualified foods has challenged food technology to produce fresh-like foods by replacing thermal treatments with alternative methods of preservation. Non-thermal processing method such as high electric field pulses, oscillating magnetic field, high hydrostatic pressure and ultrasound are receiving considerable attention for their potential to reduce the number of microorganisms in foods and extend the storage time [3].

To qualify as an alternative method, a new technology should have significant impact on quality while at the same time maintain the cost of technology within feasibility limits. Among all emerging non-thermal technologies, high intensity pulsed electric fields (PEF) is one of the most appealing technologies due to its short treatment times and reduced heating effects with respect to other technologies. High intensity pulse electric field is highly appreciated as a non-thermal food preservation technology that involves the discharge of high voltage electric short pulses through the food product. PEF technology has the potential to economically and efficiently improve energy usage, besides the advantage of providing microbiologically safe and minimally processed foods [4-6].

High intensity electric field processing involves the application of high voltage (typically 20-80 kV/cm) to foods placed between two electrodes [7]. Some important aspects in pulsed electric field technology are the generation of high electric field intensities, the design of chambers that impart uniform treatment to foods with minimum increase in temperature and the design of electrodes that minimize the effect of electrolysis [8].

Electric fields processing is effective in the inactivation of most vegetative bacterial cells. The efficacy of the process depends on several factors such as electric field intensity, pulse shape and polarity, treatment time and frequency, temperature, type and concentration of microorganisms, and the electric field operation mode [9-12].

II. EXPERIMENTAL TECHNIQUE

A. Microorganisms used as Samples

Four different types of bacteria of various shape, size and genetic makeup namely Bacillus cereus, Klebseilla pneumonia, Staphylococcus aureus and Pseudomonas aeruginosa were used.

Bacillus cereus is a gram-positive, rod-shaped, motile, beta haemolytic bacterium. Some strains are harmful to humans and cause illnesses (Fig.1). B cereus is responsible for a variety of food borne illnesses (2–5%), causing severe nausea, vomiting, and diarrheic. Bacillus food borne illnesses occur due to survival of the bacterial end spores when food is improperly cooked.

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Klebseilla pneumonia is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic, rod-shaped bacterium (Fig.2). They are found in the normal flora of the mouth, skin, and intestines.
Staphylococcus aureus is a gram-positive coccal bacterium and is frequently found in the human respiratory tract and on the skin (Fig.3). S. aureus is not always pathogenic, is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning.
Pseudomonas aeruginosa is Rod shaped; Gram-negative. It can cause food spoilage (Fig.4).It is recognized for its advanced antibiotic resistance mechanisms, and its association with serious illnesses – especially nosocomial infections such as ventilator-associated pneumonia and various sepsis syndromes.

B. Sample Preparation
Freeze dried cultures of, Bacillus cereus, Klebseilla pneumonia, Staphylococcus aureus and Pseudomonas aeruginosa were used for the experiment.
The four microorganisms were cultured according to standard American Type Culture Collection (ATCC) procedures. These were supplied by either solid (nutrient agar) or liquid (nutrient broth) culture media. The medium was first dispensed into flasks and then sterilized by autoclaving. A measured amount of bacterial suspension was poured into a petri dish and the melted nutrient agar medium at 45˚ C was added and the two were thoroughly mixed by rotating plate. When medium solidified the organisms were trapped in agar and the plates were then incubated for 24 hours or longer at 37°C in BOD Incubator. Each organism grew, reproducing itself until a visible mass of organism, a colony developed; i.e. one organism gave rise to one colony. A colony count performed on the plate revealed the viable microbial population of the inoculums.

C. Quantitative Measurement of Bacterial Growth
The Standard Plate Count (SPC) technique was used for the estimation of bacterial population in nutrient broth [13]. The SPC is the number of bacterial colonies that develop on a medium in a petri dish seeded with a known amount of inoculum. The number of bacteria in a given sample is usually too great to be counted directly. However, if the sample is serially diluted and then plated out on an agar surface in such a manner that single isolated bacteria form visible isolated colonies , the number of colonies can be used as a measure of the number of viable (living) cells in that known dilution.

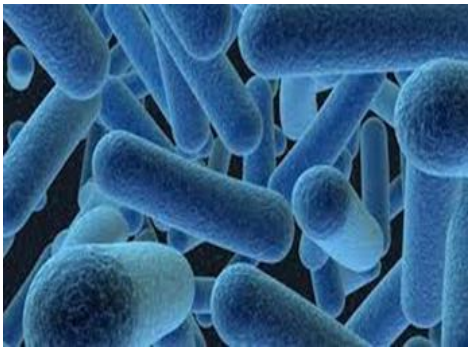

Fig.1 Bacillus cereus

The original sample containing the microorganisms was serially diluted so that the number of colonies developing on the plate fell within the range of 30 to 300.

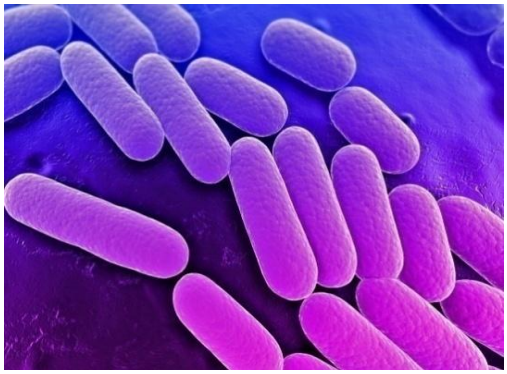

Fig.2 Klebseilla pneumonia

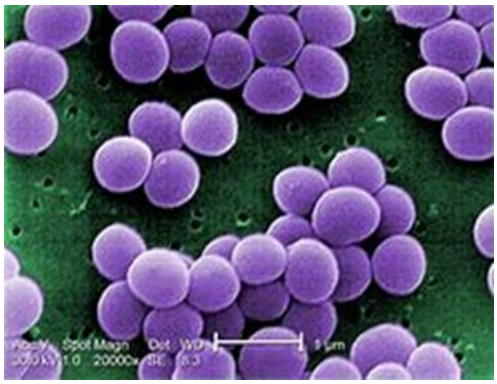

Fig.3Staphylococcus aureus

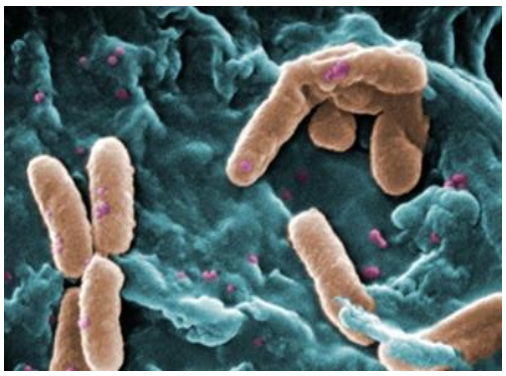

Fig.4 Pseudomonas aeruginosa

A plate having 30-300 colonies waschosen because this rangeis considered statistically significant. If there were less than 30 colonies on the plate, small errors in dilution technique or the presence of a few contaminants will have a drastic effect on the final count. Likewise, if there were more than 300 colonies on the plate, there will be poor isolation and colonies will have grown together.
A colony count performed on the plate revealed the viable microbial population of the inoculums. To determine the number of microorganisms per millilitre (ml) of sample, the number of colonies (on a plate having 30-300 colonies) is multiplied by the number of times the original ml of bacteria was diluted. The typical viable count was 10^8 colony-forming units per ml (CFU/ml) for standard sample.

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D. Treatment Chamber

The treatment chamber was made of Perspex (Fig.5). Perspex has been chosen because it lends itself readily to all types of mechanical working and is washable. Two parallel circular electrodes were mounted in the treatment chamber. Both the electrodes were made of brass and were 9 cm in diameter (Fig.6). The separation between the electrodes was 0.8 cm. They were polished, buffed and cleaned. While handlingcare was takento keeptheelectrode surfaces untouched and free from scratches, dust and other impurities. The electrodes were mounted vertically. Liquid containing bacteria in known population was poured in the treatment chamber until the electrode assembly was completely filled. Measurements were initiated by applying ac voltage of known magnitude for specified duration. The applied voltage was 1.2/50 µs impulse voltage obtained from 980 Joule, 280 kV 2-stage impulse generator (Fig.7). The voltages were measured with an accuracy of ± 3%.

E. Procedure for Microbial Inactivation

The pre-autoclaved treatment chamber was cleaned with sterile distilled water, disinfected with 70% alcohol and was allowed to dry for a period of one hour, before introducing any samples. The chamber was filled gradually with 40 ml of sample without any air bubbles and application of AC voltage was initiated immediately after closing the chamber. After each treatment, the sample was removed from the chamber and stored in ice bath to keep the temperature under control. After the removal of each processed sample, the chamber was sterilized before the next batch treatment. Two set of experiments were carried out on standard media, each inoculated with only one type of bacteria.

Fig.5 Treatment Chamber

Fig.6 Electrodes

In the first set of experiment the number of pulses was fixed at 100 and applied peak voltage was varied within a range of 20 to 80 KV. In the second set experiment the peak voltage was fixed at 80 KV and the number of pulses was varied within the range of 25 to 100. In both the set of experiments, the Bacterial growth reduction of various bacteria was observed after application of the applied PEF.

Fig.7 Impulse Generator

III.RESULTS

From the data obtained from the first set of experiments bacterial growth reduction of various microbes vs. peak voltage have been plotted for a fixed number of pulses and are shown in Figures 8-11, where

C_o = Initial concentration of Microbes.
C = Final concentration of Microbes.
Log (C/C_o) = Bacterial growth reduction

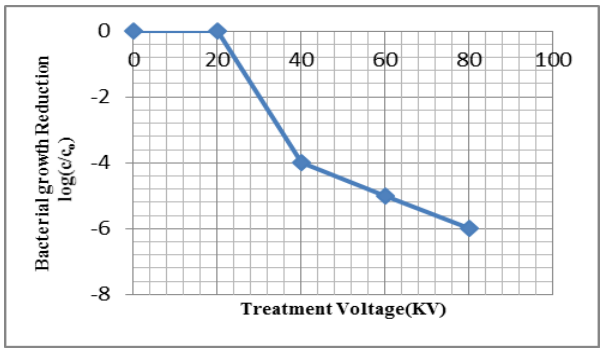

Fig.8 Growth Reduction curve of Bacillus cereus at constant number of pulses.

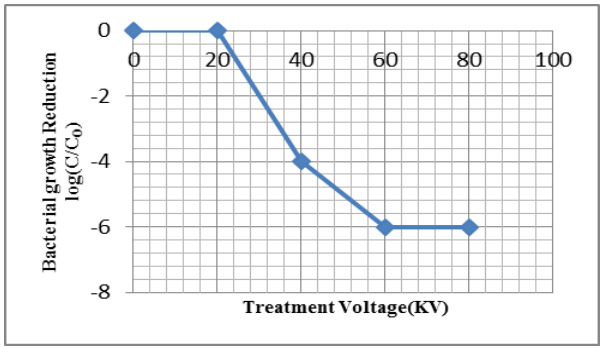

Fig.9 Growth Reduction curve of Klebseilla pneumonia at constant number of pulses.

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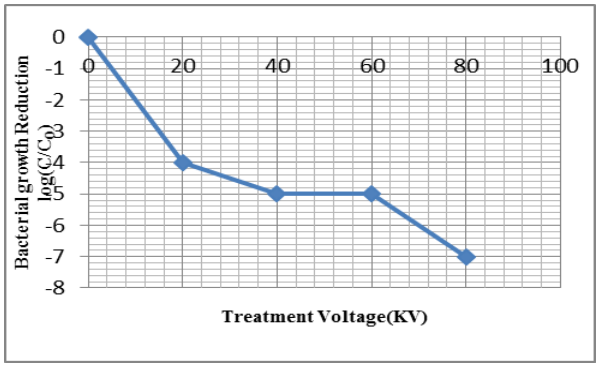

Fig.10 Growth Reduction curve of Staphylococcus aureus at constant number of pulses.

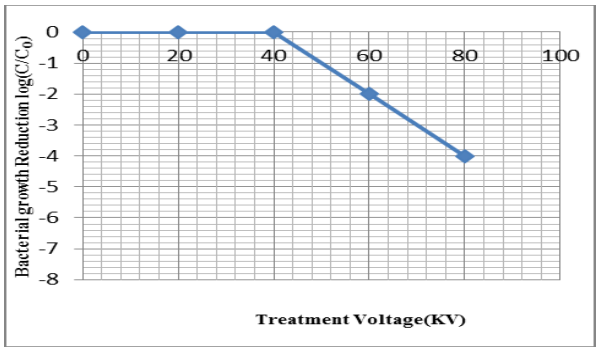

Fig.11 Growth Reduction curve of Pseudomonas aeruginosa at constant number of pulses

Similarly from the data obtained from second set of experiments bacterial growth reduction of various microbes vs. number of pulses have been plotted keeping a keeping a constant peak voltage at 80 kV and are shown in Figures 12-15.

IV. DISCUSSION & CONCLUSION

For experiments done by varying the peak of the applied impulse voltage while keeping the number of pulses constant at 100 (Fig.8-12), no destruction of Bacillus cereus and Klebseilla Pneumonia was observed up to 20 kV voltage. However, Staphylococcus aureus showed a 4-order reduction in bacterial growth when the peak applied impulse voltage was raised to 20 kV. Pseudomonas aeruginosa exhibited maximum resistance to the applied voltage and no reduction in bacterial growth was observed up to 40 kV peak of applied voltage.

When the applied voltage was further raised in steps to 80 kV, Bacillus cereus showed a 6-order reduction in bacterial growth. Klebseilla Pneumonia also showed a 6-order reduction in bacterial growth when peak voltage was raised to 60 kV after which no further reduction was observed even when the voltage was raised to 80 kV peak. Staphylococcus aureus exhibited maximum reduction in bacterial growth and showed a 7-order reduction when the voltage was raised to 80 kV peak. Pseudomonas aeruginosa continued to exhibit strong resistance to the applied voltage and showed only a 4-order reduction in bacterial growth when peak voltage was raised to 80 kV.

For experiments done by varying the number of pulses of the applied voltage while keeping the peak voltage constant at 100 kV (Fig.12-15), Bacillus cereus, Klebseillapneumonia and Staphylococcus aureus exhibited a 5-order reduction in bacterial growth when 25 pulses were applied. However, Pseudomonas aeruginosa exhibited no destruction for the same dose.

A 6-order reduction in bacterial growthwas achieved for Bacillus cereusand Klebseillapneumoniawhen the number of pulses were increased to 50 and thereafter no further reduction was observed even when the number of pulses were increased to 100. However, Staphylococcus aureus exhibited a 7-order reduction in bacterial growth when the numbers of pulses were increased to 75 and thereafter no further reduction was observed even when the number of pulses were increased to 100.

Pseudomonas aeruginosa exhibited only a 4-order reduction in bacterial growth when the numbers of pulses were increased to 50 and thereafter no further reduction was observed even when the numbers of pulses were increased to 100.

From the above results it can be concluded that a higher applied voltage will result in a larger reduction in the bacterial growth. However, no sizeable reduction in micro-organisms is observed if the numbers of pulses are increased beyond 50. Thus an optimum dose of PEF voltage magnitude and number of pulses for treatment of the food infected with microorganisms can be determined. However a more comprehensive study is desired to determine optimum dosage for various microorganisms using PEF technology before it can be put to commercial use.

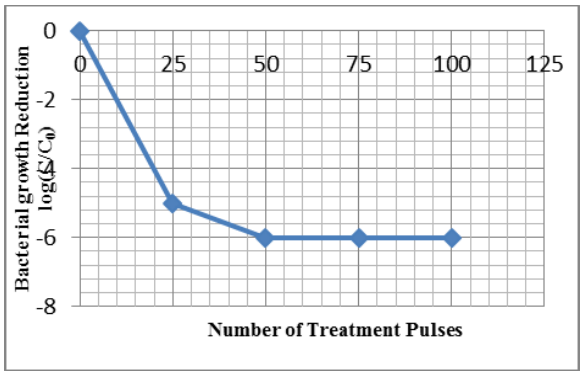

Fig.12 Growth Reduction curve of Bacillus cereus at constant voltage

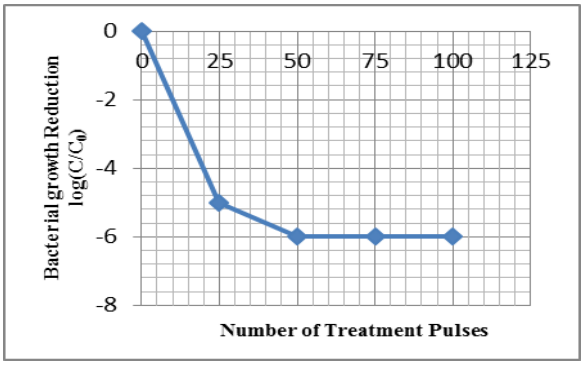

Fig.13 Growth Reduction curve of Klebseilla pneumonia at constant voltage

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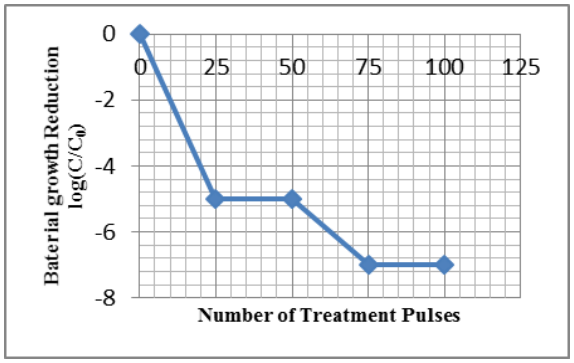

Fig.14 Growth Reduction curve of Staphylococcus aureus at constant voltage

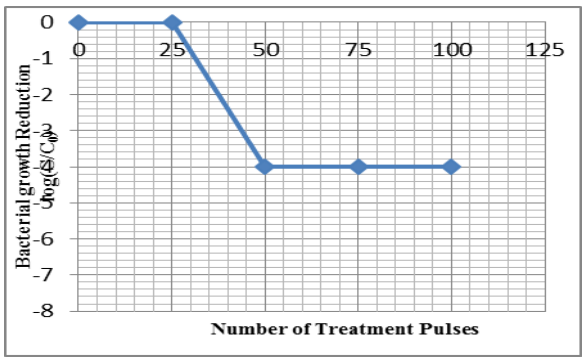

Fig.15 Growth Reduction curve of Pseudomonas aeruginosa at constant voltage

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