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NANOSECOND PULSED ELECTRIC FIELD INHIBITS GROWTH IN PSEUDOMONAS AERUGINOSA

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ABSTRACT

Objective: Nanosecond pulsed electric fields (nsPEFs) have great potential for modern biotechnological and new medical applications. This paper shows the effect of nanosecond pulsed electric field (nsPEF) on Pseudomonas aeruginosa, which could imply a durable change in protein expressions of surviving bacteria that might lead to increase pathogenicity. **Methods and Results:** The effects of nsPEF with Electric pulse duration of ($60x10^{-9}$s) with a rise time of ($20x10^{-9}$s), a high voltage of ($2x10^{4}$V) and a repetition frequency of (0.5Hz) on bacteria viability. One log10 reduction in bacterial counts was achieved at field strength of (10^{7} V m^{-1}) with a train of 500 successive pulses of ($60x10^{-9}$s). To observe a possible change in Pseudomonas aeruginosa, studies were performed for differential protein expressions using gel electrophoresis (SDS-PAGE). As all the CFU were not investigated by this method, antibiograms were used to a larger extent to show that after the treatment, antibiotics were still able to neutralize these germs and if not, the nsPEF could act on naked DNA by expressed proteins that might reduce the sensibility of the bacterium by overexpressing or modifying the tridimensional structure of the target for a given antibiotic. The former frequency enhances the microorganism growth by 29 % and the DNA structure of the bacteria didn't change, while the later frequency caused inhibition in the microbial activity by 52% and pronounced changes in the DNA structure occurred. **Conclusions:** The results tend to show that nsPEFs are able to inactivate bacteria and have probably no serious impact in Pseudomonas aeruginosa protein patterns.

KEYWORDS: nsPEFs - Pseudomonas aeruginosa–antibiotic.

INTRODUCTION

Application of pulsed electric field (PEF) has been found as a new treatment for food preservation where short electrical pulses are applied momentarily to samples through conductive electrodes in direct contact with samples. Major portion of research efforts on PEF has been focusing on a reduction of microbial fractions in liquid food (S. F. Aguilar *et al.*, 2007) (P. Manas *et al*., 2001) or semi-solid food (D. J. Bolton *et al*., 2002). The application of nsPEF with a pulse width at electric field of ($20x10^{6}$ Vm^{-1}) and half maximal of ($80x10^{-9}$s) also showed increased phosphorylation of c-jun protein with increased c-jun and c-fos mRNA levels (Morotomi-Yano *et al.*, 2011a,b).Moreover, PEF treatment preserves nuclease activity, providing enzymatic degradation of DNA (Rieder *et al.*, 2008). This can considerably reduce the spread of resistance plasmids originating from antibiotic-resistant bacteria in clinical wastewater (Poyatos *et al.*, 2011) .Despite all its advantages, PEF disinfection has to compete with conventional techniques from an economic point of view (Poyatos *et al.*, 2011). Nanosecond pulsed electric fields (nsPEF) are a promising technology to increase the viability of microalgae cultivation systems. Among the possible applications of nsPEF are sub-lethal stress induction as well as targeted microbial flora control (Buchmann *et al*., 2018b).In nsPEF applications on eukaryotic cells, an increased growth was observable after the treatment (Eing *et al*., 2009; Gusbeth *et al*., 2013).

The use of nsPEF is a safe promising new non thermal method for bacterial inactivation in the environmental industry and food processing .Several years ago the high-power electrical modern technologies were able to generate a new type of pulsed electric field (PEF) is called nanosecond pulsed electric field (nsPEF). (MacGregor *et al.*, 2000; Rowan *et al.*, 2000).The characterized nanosecond pulsed electric field (nsPEF) are very short pulse duration (few tens of nanoseconds), a high electric field intensity (in the range of several hundreds of thousands (Vm^{-1}) and (nsPEF) a delivered energy low enough to avoid heating effect. (Chen et al., 2004; Pakhomov *et al.*, 2007; Dalmay *et al.*, 2011; Nesin *et al.*, 2011).These nsPEFs were used to inactivate bacteria in liquid.(Schoenbach *et al.*, 2000). Some study showed that nsPEF could induce an injury accumulation

in survival bacteria leading to subsequent death (Perni *et al.*, 2007; Gusbeth *et al.*, 2009).The nsPEFs acting directly at the DNA levels or protein by inducing the denaturation and/or the destruction of these molecules (Stacey *et al.*, 2003, 2011). This process causes structural damage to microbial membranes at less significant energy levels when compared to typical heating process. As part of the response to an increasing voltage across samples, the resultant transmembrane potential causes the cell membrane to lose its impermeability beyond the critical value, owing to the pore formation induced on the membrane surface leading to the disintegration of the cell membranes.[8]-[9]

The strength of the electrical field that passes through the food is directly proportional to the voltage supplied across the electrodes, and inversely proportional to the distance between the electrodes. Currently, the two mechanisms of necrosis and apoptosis have been suggested for cell destruction (Stephen, J., Beebe Nova *et al.*, 2013). The use of high pulse electrical fields (HPEF) for microbial inactivation in liquid food is attracting the attention of both academic researchers and food investors (Rittipun *et al.*, 2014).

AIM OF THE WORK

This paper a microbial reduction as results of nsPEF treatments using laboratory-assembled batch treatment chamber. we report the effects of nsPEF with Electric pulse duration of (60 $x10^{-9}$s) with a rise time of ($20x10^{-9}$s), a high voltage of ($2x10^{4}$V) and a repetition frequency of (0.5Hz) on bacteria viability. In results observe a possible change in Pseudomonas aeruginosa, studies were performed for differential protein expressions using gel electrophoresis (SDS-PAGE) As all the CFU were not investigated by this method, antibiotics were still able to neutralize these germs and if not, this will highlight that nsPEF could act on naked DNA by expressed proteins that might reduce the sensibility of the bacterium by modifying the tridimensional structure of the target for a given antibiotic.

MATERIALS AND METHODS

1-Nanosecond pulsed electric field (nsPEF)

The shape of the delivered voltage pulse is shown in Figure (1). The experimental setup consists of a pulse generator, a voltage and current diagnostic system, and an optical stage for accurately positioning bacteria culture in petri dish plate. The nsPEF device delivery system was composed of a high-voltage generator and a chamber of treatment. Electric pulse duration of ($60x10^{-9}$s) with a rise time of ($20x10^{-9}$s), a maximal voltage of ($2x10^{4}$V) and a repetition frequency of (0.5Hz). Square Amplitude Modulated Waves (QAMW), generated from two generators. The system fabricated and manufactured locally in the physics lab of October 6 University in Cairo-Egypt as shown in figure-1.

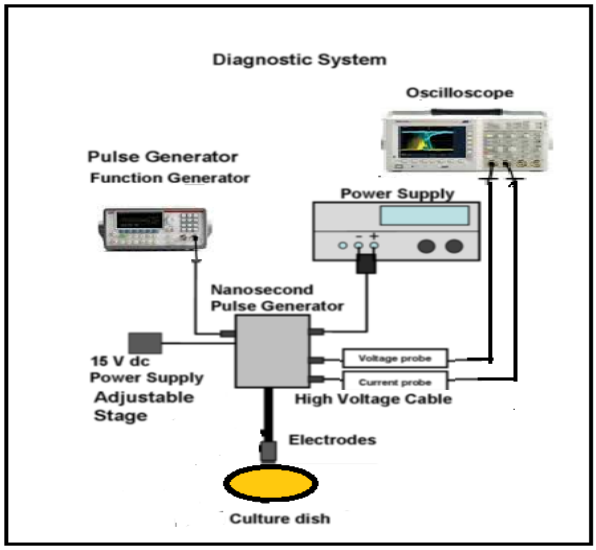

Figure (1): Schematic of the experimental setup for nanosecond pulsed electric field (nsPEF) mapping.

2- Pseudomonas aeruginosa samples

Reference strain of Pseudomonas aeruginosa (Ref 0353P, ATCC 27853, LOT 353604, OXOID) is used for all the present comparative experiments. The microorganism is plated on MacConkey agar and incubated at 37ºC 24 hrs. Thereafter, several colonies plated again on a fresh agar plate and also incubated for 24 hrs at 37^{0}C. For maintaining a fresh strain, this procedure is repeated weekly before running the experiment.

3-Determination of Bacterial Replication

The bacterial growth of Pseudomonas aeruginosa is determined by measuring the optical density (OD) at 700 nm (the best wave length for absorbance of bacterial suspension that selected automatically) using spectrophotometer type (JENWAY 6405 UV/VisibleU.K.). A calibration curve between the OD and cell count was established by preparing a bacterial suspension which was produced by suspending three colonies from fresh 24 hrs incubated plated agar of Pseudomonas aeruginosa in 5 ml of MacConkey broth media and then incubated for 12 hrs at 37ºC (the experimental time range). Every 60 min intervals within the time range the OD measured and different dilutions of each sample are plated on MacConkey agar. After the incubation for 24 hrs at 37ºC of the plates, colony forming units CFU are counted and then correlated to the OD and its values plotted versus CFU/ml values and a best fit line is performed. Then the growth characteristic curve for each sample could be plotted between the CFU/ml and time directly (Obermeier *et al.*, 2009). It is worthy to mention that the absolute reference values of CFU were being differed from experiment to another due to the various amounts of bacterial suspension used to inoculate the agar plates. However, within the one experimental setup the CFU were identical for the exposed and reference samples. Moreover, the check of the concentrations was applied by keeping the

absorbance almost the same for all samples before running the experiment.

4-Inhibition Frequency Determination

Pseudomonas aeruginosa sample exposed to ($60x10^{-9}$s) with a rise time of ($20x10^{-9}$s), a maximal voltage of ($2x10^{4}$V) and a repetition frequency of (0.5Hz). A fresh bacterial suspension of it was prepared as mentioned before. The concentration of the suspension has been adjusted to approximately 10^{7} CFU/ml by using the OD as mentioned before. The Pseudomonas aeruginosa suspension then divided into two groups (three samples per each), one group kept as a control (unexposed) group and the other group exposed to nsPEF source where the frequency (0.5Hz) in ($60x10^{-9}$s) with a rise time of ($20x10^{-9}$s), a maximal voltage of ($2x10^{4}$V).At the end of the exposure period, the bacterial growths for both the control and exposed groups were determined in parallel by measuring the absorbance at wavelength 700 nm. Then, the sample groups are incubated at 37ºC and every 1 h the incubation are interrupted for absorbance measurements and different dilutions of each sample is plated on MacConkey agar in order to determine its cellular counts to detect the resonance frequency that cause maximum growth inhibition. The inhibition percentage difference in 10^{th} hour incubation for each sample is calculated with respect to its control one according to equation-1 and the average value is taken. Then the average inhibition percentage difference values are plotted versus frequency in the (0.5Hz).

$$(\%D) = \frac{\text{Average OD (control)} - \text{Average OD (exposed)}}{\text{Average OD (control)}} \text{x100}$$

After the end of the exposure time, all groups are incubated and then absorbance measurements were done every 1 hr. The average percentage inhibition value (as compared to control) at 10^{th} hr post incubation for each group was calculated to specify the most effective time.

5-Electrostatic calculation of (nsPEF) distribution

Electric pulse duration of ($60x10^{-9}$s) with a rise time of ($20x10^{-9}$s), a maximal voltage of ($2x10^{4}$V) and a repetition frequency of (0.5Hz) .The skin depth, d and the wavelength, λ, of an electromagnetic wave propagating in a medium with zero magnetic susceptibility (relative permeability = 1) depend on the dielectric constant, ε r and on the frequency, f, and conductivity, σ, of the growth medium, as in the following equations (2) and (3):

$$\lambda = \frac{2\pi}{\frac{\omega}{c}\sqrt{\frac{\varepsilon_r}{2}\left[\sqrt{1+\left(\frac{\sigma}{\varepsilon\omega}\right)^2}+1\right]}^{1/2}}$$

$$d = \frac{1}{\frac{\omega}{c}\sqrt{\frac{\varepsilon_r}{2}\left[\sqrt{1+\left(\frac{\sigma}{\varepsilon\omega}\right)^2}-1\right]}^{1/2}}$$

Where ω = 2πf is the angular frequency ,*c* is the speed of the light in a vacuum, , ε is the permittivity of free space (ε = 8.85 x 10^{-12} F/m) ,and conductance (σ) .In case of an applied alternating field, the dielectric properties (ε, σ) will vary with frequency (Vernier PT, *et al.*, 2003).

6- Antibiotic Susceptibility Test

Antibiotics had diffused in the solid culture medium forming a gradient of concentration. If bacteria were sensitive and killed by antibiotic, a lyzed zone was observed. If bacteria became resistant for an antibiotic, the lyzed zone would not be present or had a smaller size.10% of the CFU growing in all Petri dishes used in the tests (in the limit of 10 CFU petri dish maximum) was picked out for antibiogram assays. Each CFU was incubated in LB broth medium. When stationary phase was reached, culture was spread on a Petri dish at high concentration. Six antibiotics were used: trimethoprim associated with sulphamethoxazole (SXT), gentamicin (GM), erythromycin (E), penicillin (P), ceftazidine (CAZ), and vancomycin (VA), six discs soaked with one of each antibiotic were put on Petri dish. After incubation overnight at 37°C, a film of bacteria was formed.

7-SDS Gel electrophoresis

The two-dimensional electrophoresis was first carried out with an isoelectro -focusing (IEF) followed by a sodium dodecylsulphate-gel electrophoresis (SDS-PAGE) as previously described (Joubert *et al.*, 2013). The pattern of water- soluble proteins was determined by isoelectric focusing (IEF) and sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) after 24 h exposure to nsPEF. Incubation of the bacteria was performed in medium at different temperatures from 37°C to 43 °C. IEF was carried out in the pH range 3-10, SDS-PAGE was performed, using a 10% separating gel and a 4% storage gel, following the instructions of (Joubert *et al.*, 2013).

8-Plasmid Analysis of Pseudomonas aeruginosa isolated strains Plasmid preparation

This method has been used for the isolation of plasmids from gram-negative bacteria and gram-positive bacteria. It is recommended for the routine screening of all types of bacteria for all types of plasmids. Isolation of plasmids from Pseudomonas aeruginosa was done by the boiling method according to Holmes and Quigley, 1981.Two ml of bacterial salt medium were inoculated with the tested microbial cells and incubated over night at (37ºC) with shaking. The cultures were then diluted to 1:20 in (2 ml) LB broth medium and incubated for (2 to 3 hr). The irradiated and non-irradiated bacterial cells were harvested and the cell pellets were re-suspended in

(1 ml) STET buffer (sucrose, Tris-Hcl, Triton x 100 and ethylene diamine tetraacetic acid (EDTA) (0.025 M) then mixed well with (90 µl) lysozome and incubated on ice water for (30 min). They were incubated for 1 min in boiling water bath then centrifuged at (14,000 rpm) for (20 min). The supernatant was transferred to clean centrifuge tubes. In each tube 0.6 ml of cold isopropanol was added to the supernatant fluid, the tubes were mixed and incubated in ice water for (20 to 30 min). Then were centrifuged for (20 to 30 min) at (1400 rpm). Supernatant was poured off and the tubes were inverted on a paper towel then dried for (2 min) under vacuum. The precipitate was resuspended in (100 µl) of TE buffer (1 M Tris HCl – 0.5 M EDTA, pH: 8), incubated over night at (+4ºC) to allow the plasmid DNA to dissolve, and analyzed by agarose gel electrophoresis.

9-Agarose Gel Electrophoresis for isolation of plasmid DNA

Electrophoresis of plasmid DNA was done on a horizontal gel apparatus.Agarose (1%) in 1xTris-base (0.89 M) buffer was prepared. Ten micro liters of plasmid DNA and 2 µl of loading buffer were mixed well and loaded into the gel containing (10µl) ethidium bromide (1µg/ml in water) and the electrophoresis was conducted for 90-120 min at constant voltage 75V. In Tris-borate buffer according to method of (Meyers *et al.*, 1976). Trans-illuminator at wavelength (312 nm) examined the gel. The data obtained from the scanning process of each gel were analyzed using progel (Image master ID gel analysis v 3.0), to determine the degree of similarity and dissimilarity between the plasmid profile of the different tested isolates before and after exposure to the fields.

10-Statistical analysis

All tests were performed at least in triplicate. One way analysis of variance (ANOVA-test) was performed by Graphpad Instat for testing of differences between two groups. $P < 0.05$) was considered to be statistically significant.

RESULTS

1- Growth Curve Characteristics

Figure (2) shows the variation of sample optical density (OD) with Pseudomonas aeruginosa cell count in MacConkey broth medium measured at (700 nm) indicated a linear dependence of the absorbance on the microorganism count according Bioscience).The relationship shows linear dependence of the absorbance on the CFU with a slope ($2x10^8$).

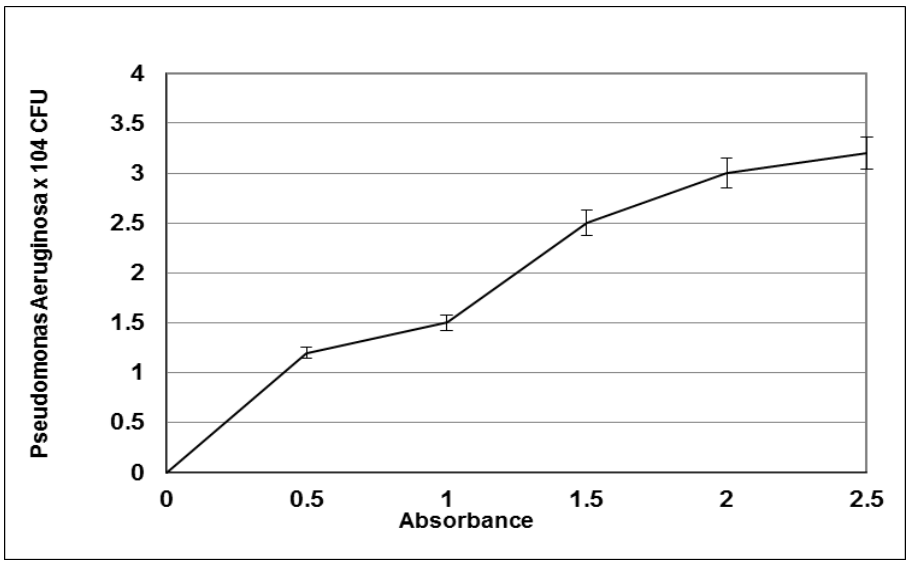

Figure (2): The calibration relationship for CFU of Pseudomonas aeruginosa as a function of absorbance.

2-Effect of nsPEF deliveries on Pseudomonas aeruginosa viability

Figure (3) shows the results obtained a log10 decrease was obtained after 500 nsPEFs at a frequency of (0.5Hz). Pseudomonas aeruginosa suspensions were exposed to various numbers of nsPEF pulses with an electric field of ($10^7 Vm^{-1}$) and a repetition frequency of (0.5Hz). Electric pulse duration of ($60x10^{-9}$s) with a rise time of ($20x10^{-9}$s), a maximal voltage of ($2x10^4$V) and a repetition frequency of (0.5Hz). Pseudomonas aeruginosa sensitive to nsPEFs, and inactivation was statistically noticeable for 100 and 500 nsPEF pulses compared with (P values ≤ 0.05 and ≤0.01), respectively. The results indicate remarkable a decrease of (54%) at (500 nsPEF) pulses relative to control for those exposed to frequency (0.5Hz). Fluctuations in the number of cells dependence on higher frequencies but with lower intensities.

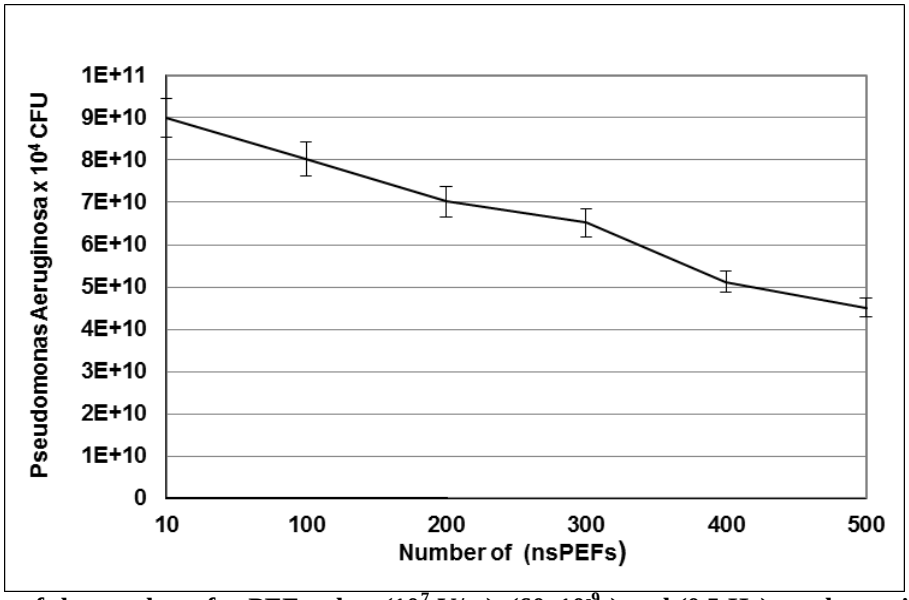

Figure (3): Effect of the number of nsPEF pulses (10^7 V/m), ($60x10^{-9}$s) and (0.5 Hz) on change in the number of cells (CFU) Pseudomonas aeruginosa.

2-Effect of nsPEFs in response to antibiotics

Figure (4) showed the inhibitory effect of application of nsPEFs to Pseudomonas aeruginosa several colonies of treated (500 pulses) or untreated germs being able of survival or recovery with growing capacity were taken for antibiogram purpose. Diameters of inhibition were measured parallel to the border of the Petri dish (arrow) and performed in the same way for of all the antibiotics. It was possible to determine if the bacteria were resistant (R), sensitive (S) or intermediary (I) to the action of antibiotics used according to the Pasteur Institute recommendation. For CAZ, the control was (I), and after nsPEF, it was found to be (S/I), and for the other antibiotic, it passed from (S/I) to (I). Antibiogram of Pseudomonas aeruginosa with six different antibiotics: vancomycin (VA), ceftazidine (CAZ), penicillin (P), gentamicin (GM), erythromycin (E), trimethoprim associated with sulphamethoxazole (SXT), and. Each bar corresponds to 45 takings of five independent experiments. Fig (3) shows the changes in the difference from control was no significant differences are noticeable for a given antibiotic between the samples and the control. R means resistance to the antibiotic, I for intermediate and S for sensitive ($p < 0.05$).

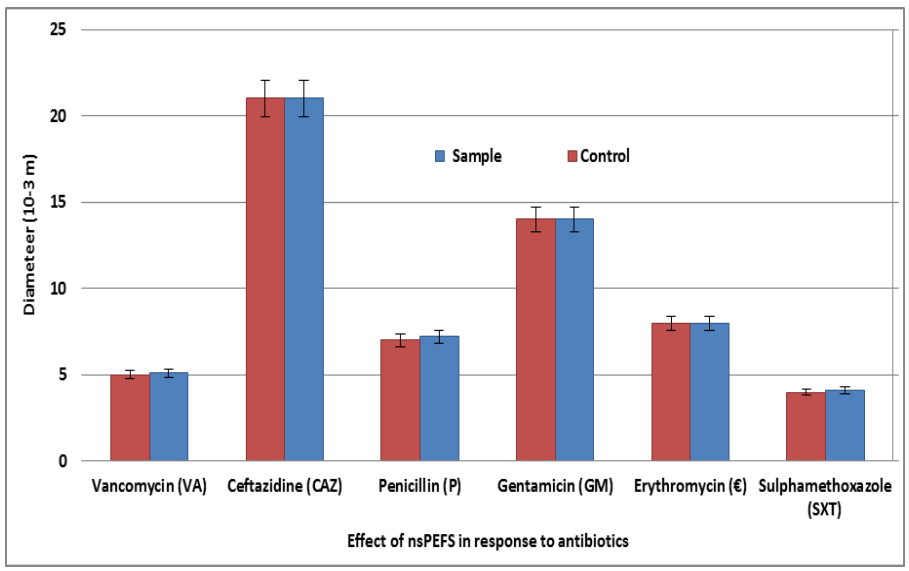

Figure (4): Antibiogram of Pseudomonas aeruginosa with six different antibiotics.

4-Effect of nsPEFs on protein expression

Figure (5) showed the nsPEFs (10^7 V/m), ($60x10^{-9}$s) and (0.5Hz) were able to induce differential protein expression due to the high electric field towards the bacteria. Pseudomonas aeruginosa are known to produce stress proteins, e.g. induced by heat (Haemmerich D, *et al.*, 2005) .Fig (5-A&5-B) showed SDS-PAGE was performed on CFU treated or not by nsPEF respectively. SDS-PAGE showed similar results. SDS-PAGE between the control and Pseudomonas aeruginosa treated with

500 nsPEFs, only few changes in protein expression were present but only one of all the sets presented a differential expression, Pseudomonas aeruginosa .

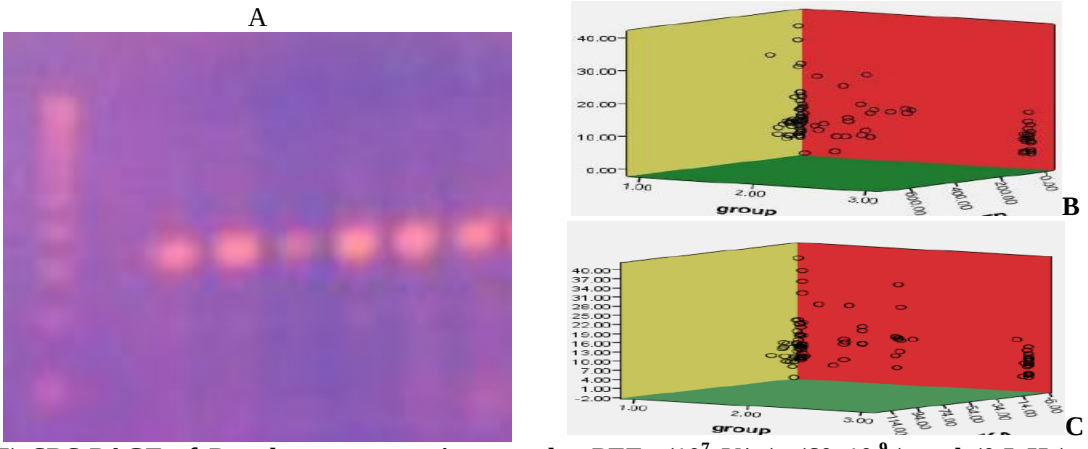

Figure (5) SDS-PAGE of Pseudomonas aeruginosa and nsPEFs (10^7 V/m), ($60x10^{-9}$s) and (0.5 Hz) effect on protein expression (A). Protein expression at Pseudomonas aeruginosa control group (B). nsPEF slightly significantly increased protein expression at Pseudomonas aeruginosa control group (C).

5-Plasmid analysis

The gel was read out and the results for each lane are shown in figure (5). It is clear from the figure that there is structural change in the molecular structure of the plasmid DNA extracted from exposed Pseudomonas aeruginosa to nsPEFs (10^7 V/m), ($60x10^{-9}$s) and (0.5Hz). This can be easily noticed from figure (6) as compared with figure (7). Figure (6) shows the electropherogram corresponding to the scanned gel of plasmid DNA extracted from Pseudomonas aeruginosa. Figure (7) shows the electropherogram corresponding to the scanned gel of plasmid DNA extracted from Pseudomonas aeruginosa after exposure to nsPEFs (10^7 V/m), ($60x10^{-9}$s) and (0.5Hz).

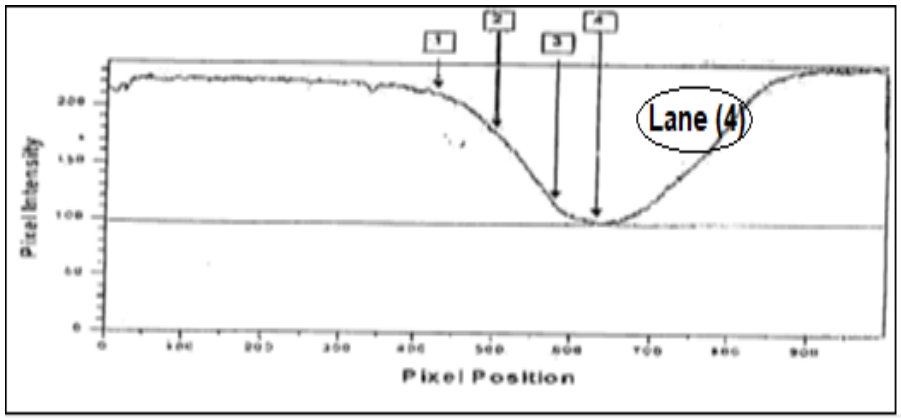

Figure (6): The electropherogram corresponding to the scanned gel of plasmid DNA extracted from control Pseudomonas aeruginosa.

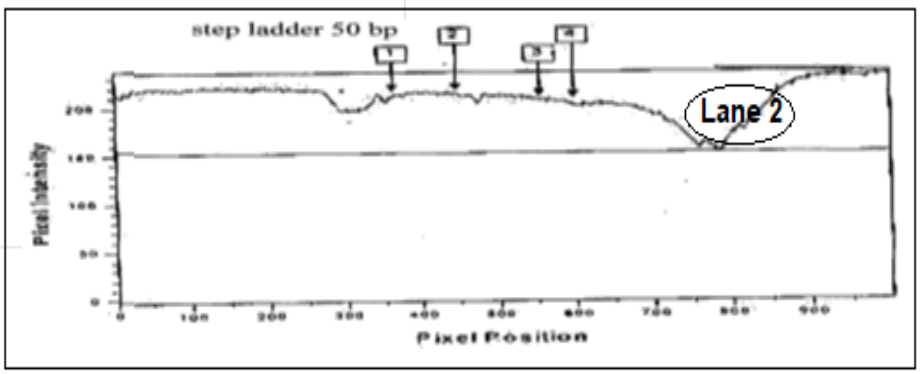

Figure (7):The electropherogram corresponds to the scanned gel of plasmid DNA extracted from Pseudomonas aeruginosa after exposure to nsPEFs (10^7 V/m), ($60x10^{-9}$s) and (0.5 Hz)

DISCUSSION

In the present work the control of Pseudomonas aeruginosa. In activity by nsPEF was studied. The procedure of treatments of the microorganism was based on the resonance interference of applied electric fields with the bioelectricity signals generated during cellular division. Our data suggest that nsPEF had direct effects on Pseudomonas aeruginosa, including the inhibition of growth culture .The nsPEF deliveries on Pseudomonas aeruginosa suspension induced a bacterial inactivation (around one log10 after 500 pulses). (Fadel 1998, Fadel *et al.*, 2008), to interfere with biological electrical signals generated during metabolic activities or in activity of cells it is necessary to apply on these cells external electric waves of the same frequency. (Pothakamury *et al.*, 1996) illustrated that square amplitude magnetic waves (QAMW) pulses were more lethal than exponentially decaying pulses. It is well known also that bioelectric signals generated during metabolic activities of cells are in the extremely low frequency range (Patton el al. 1989) (Fadel 2005, Fadel *et al.*, 2010) .The applied electromagnetic wave should have the same frequency of the bioelectric signal. Since the electrical impedance of tissues ($X_{C=}1/ (2\pi fc)$) is inversely frequency (f) dependent, and will be equal to infinity for direct current (D.C), it was necessary to use amplitude modulated waves, with wave carrier of higher frequency to have lower tissue impedance for the applied electromagnetic wave.

In this work a wave carrier of nsPEF pulses (10^7 V/m), ($60x10^{-9}$s) and (0.5 Hz) on change in the number of cells (CFU) Pseudomonas aeruginosa was used. The results indicated that the Pseudomonas aeruginosa has frequencies with the bioelectric signals generated during cellular division. The frequency is at (0.5Hz) as was shown in figure (3). The frequency effect is inhibiting the cellular division.

Pseudomonas aeruginosa is known as one of the most aggressive microorganisms because of its toxicity to biological tissues and its resistivity against almost all known antibiotics. The toxins secreted by Pseudomonas aeruginosa can destroy cellular structure. On the other hand after nsPEF applications, transient over expressions of proteins or enzymes that might act at the DNA level might occur, Pseudomonas aeruginosa after several generations were able to recover almost a normal pattern. (Ramsey *et al.*, 2004; Chang *et al.*, 2010). SDS-PAGE electrophoresis and proteomic experiments showed that only one protein was still overexpressed after 24 h of multiclone proliferation, suggesting that the treated cells were not affected or that repair, if the DNA was damaged, was still effective, as was shown in figure (5) However, studies on mammalian cells showed that strong pulsed electric fields might affect DNA configuration (Stacey *et al.*, 2003, 2011). This has already been described in other biological models (Nesin *et al.*, 2011).

In this work, the slight differences observed between the treatment and the control of the protein pattern might also suggest a good recovery among time of these bacteria after the treatment or no changes at all. This study showed that ($60x10^{-9}$s) long nsPEFs had an impact on membrane permeation and nsPEFs were able to decrease viable Pseudomonas aeruginosa by one decimal magnitude after 500 pulses. The treatment showed only minor changes and might be an interesting and safe way to deactivate or destroy bacteria in waste water at low energy cost. However, it remains to determine to what extent this technology enables to treat large volumes, in accordance with an industrial process. These results may indicate that there is destructive interference of the (0.5 Hz) nsPEFs with the bioelectric signals generated during the natural micro-molecular movements within the DNA macromolecule which may be the causal factor for these changes. Abiotic sub-lethal stress induction via nanosecond pulsed electric field (nsPEF) treatment might be a viable process to increase the efficiency of photoautotrophic microalgae cultivation. (Leandro Buchmann *et al.*, 2019).

However nsPEFs stressed these mammalian cells by acting on the AMP activated protein kinase (AMPK). AMPK activation by nsPEFs was mediated by Ca MKK and required extracellular Ca^{+2}. Therefore, an experimental evidence for a direct link between activated cellular signalling and Ca^{+2} mobilizations in nsPEF-exposed cells was established (Morotomi-Yano *et al.*, 2012). Thus, nsPEFs can have a physiological impact in mammalian cells. At the microorganism level, it was shown on Pseudomonas with the use of 10 square pulses with ($10^7 Vm^{-1}$) electric field amplitude and submicrosecond ($0.6x10^{-6}$s) pulse duration that neither mutagenicity nor genotoxicity was found due to the treatment in hospital waste water or tap water (Gusbeth *et al.*, 2009). More work is needed to assess the significance of continuous-flow treatment concepts and of synergies between PEF treatment and other methods of microbial decontamination, e.g., thermal heating (Alkhafaji and Farid 2007; Saldana *et al.*, 2011; Sepulveda *et al.*, 2005;Toepfl *et al.*, 2007), for increasing inactivation efficiency. These nonchemical methods showed bacterial decontamination without side effects. Plasmid DNA studies indicated pronounced changes in the DNA structure for samples exposed to 0.5 Hz nsPEFs as compared with Control. This result may indicate that there is destructive interference of the (10^7 V/m), ($60x10^{-9}$s) and (0.5 Hz) on change in the number of cells (CFU) Pseudomonas aeruginosa with the bioelectric signals generated during the natural micro-molecular movements within the DNA macromolecule which may be the causal factor for these changes as was shown in figure (6 and 7).

However, it is now established that nsPEF ($300x10^{-9}$ s) for micro-organisms (Beebe *et al.*, 2012) and ($0.5x10^{-6}$ s) pulse can be considered as sub microsecond events. Exposures to such extremely nsPEFs seems to be safe

from the point of view of environmental exposures to nsPEFs electric fields since almost all international organizations dealing with the environmental non-ionizing radiation safe limits of exposures to nsPEFs indicated fields lower than 1 KV/m are safe. The fact that bacteria could not be inactivated by applying nanosecond-level pulses is in agreement with the findings of recent experiments (Zgalin *et al.*, 2012). The treatment conditions applied in the ms range (0.6 kV/cm; 40 ms) and the µs range (6 kV/cm; 150 µs) increase the betanines extraction yield (BEYmax) by 6.6 and 7.2 times, respectively as compared with the control (Luengo E *et al.*, 2016). There are minimal modifications of the metabolism heat and metabolites concentrations when 100 V/cm was applied (Dellarosa N *et al.*, 2016). (Deepika Kohli1 and N. C. Shahi 2017).

CONCLUSION

The pulsed electrical field technology is an emerging technology for food processing. In this work a wave carrier of nsPEF pulses (10^7 V/m), ($60x10^{-9}$s) and (0.5 Hz) on change in the number of cells (CFU) Pseudomonas aeruginosa was used. The results indicated that the Pseudomonas aeruginosa has frequencies with the bioelectric signals generated during cellular division.

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