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EFFECT AND MECHANISM OF ACTION OF NON-THERMAL PLASMA IN THE SURVIVAL OF *Escherichia coli, Staphylococcus aureus* AND *Saccharomyces cerevisiae*

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ABSTRACT

Non-thermal plasma (NTP) is an emerging technology of great interest because the elimination of pathogens and spoilage microorganisms and their probable use in various industrial sectors such as food and health. In this study was to evaluate the inactivation of non-thermal plasma on the survival of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 51811 and *Saccharomyces cerevisiae*. Treatments of different microorganisms were conducted with cells exposed NTP generated dielectric barrier reactor (DBD) able to produce atmospheric pressure plasmas. These devices operate at 13.56 MHz and are driven by a specifically built radio frequency (RF) resonant converter. The reactors, which operate at atmospheric pressure in a He-air gas mixture at a 1.5 L min^{-1} flow rate, applying a 850 V voltage at different exposure times (10, 20, 40, 60, 80, 100 and 120 s). All the strains showed a reduction in viability of < 0.001 log CFU mL^{-1} after to exposed to plasma. The cells showed membrane damage, these effects being the main cause for microbial inactivation, caused by the action of Non-thermal plasma, which leads to membrane and/or cell wall breakdown with consecutive DNA denaturation, these being the main factors inducing microorganism death.

Keywords: Non-thermal plasma, DNA damage, cell wall, membrane, microorganisms, food safety

INTRODUCTION

The non-thermal plasma is an emerging technology that is drawing attention in different sectors of process such as food (**Bermúdez *et al.*, 2013; Yong, H. I. *et al.* 2015**) and health (**Fridman, G. *et al. 2008***), for the removal of microorganisms that degrades substrate, causing diseases and economic losses for the industry; in this sense, the control and elimination is an important challenge (**Van Houdt 2010; Skandamis 2012**). The impact of modern technologies leading to the production of minimally processed foods, both safe and of nutritional quality after the process is relevant to the food industry (**Floros 2010**). That is why in recent years new technologies have been developed to replace physical methods characterized by using heat as a primary mode to inactivate or kill microorganisms (**Knorr 2011**).
In physical sciences, "plasma" refers to the forth state of matter. The plasma permits a flux of various active uncharged species of atoms and molecules as well as UV. These active uncharged species generated in plasma will typically include ozone (O_3), NO, OH radicals, etc. These charges may consist of both electrons as well as positive and negative ions. specifications permit the disinfection or sterilization of thermosensitive materials and allow in vivo applications, opening a new and larger spectrum of possible applications. The first devices developed have already proven their bactericidal properties in vitro, ex vivo, and in vivo (**Klämpfl *et al.* 2012**). **Akishev *et al.* (2008)** worked on Atmospheric Pressure Cold Plasma APCP/ NTP sterilization and they studied *Escherichia coli* and *Serratia marcenses*, classified as prokaryote Gram (-) bacteria as well as Gram (+) bacteria *Bacillus subtilis*. They also studied the eukaryotic fungi *Candida lypolitica* and *Aspergillus niger*. They explain that the elucidation of the most probable inactivation mechanisms is dependent on several considerations: 1) NTP active agents (O, OH, O_3) are not obstructed in their action on any one particular cell by other cells present in the colony; 2) cell damage is due to active plasma agents inflicting chemical injury on cell structure; 3) damage to one cell implies no lethal consequences, but amassed injury may inactivate the cell at the time of NTP or later on; 4) mathematical analysis describing NTP microorganism inactivation points to cell wall and membrane disruption by NTP leading to DNA and cell content destruction; and finally 5) gas pressure plays a crucial role in total cell inactivation.

The effectiveness of non-thermal plasma to eliminate different pathogens has been studied by **Perni (2008),** who used non-thermal plasma decontamination on mango (*Mangifera indica*) peels and melon (*Cucumis melo L*) shells, reducing 3 log $CFUcm^{-2}$ *Pantoea agglomerans* and *Gluconoacetobacter liquefaciens*strains after 2.5 s of treatment, while to eliminate *Escherichia coli*, it was necessary to apply plasma treatment for 5 s; for *Saccharomyces cerevisiae*, treatment was longer (10 and 30 s for its inactivation on mango peel and melon shell, respectively).
Niemira and Sites (2008) studied inactivation of *Salmonella* and *Escherichia coli* O157: H7 in Golden apples and oranges. They inoculated 100 µL (~10^8 CFU) *Escherichia coli* on the surface of the apples and *Salmonella* on orange surfaces, observing that a 3 min treatment with plasma reduces the *Salmonella* population from 3.7 to 2.9 log CFU mL^{-1} and from 3.6 to 3.4 log CFU mL^{-1} for *Escherichia coli* O157:H7. Similarly, **Gallagher *et al.* (2007)** investigated the effect of non-thermal plasma on air decontamination, obtaining up to 5 Log mL^{-1} reduction of *Escherichia coli* (strain K-12). A similar result was obtained by **Vaze *et al.* (2010)** who observed up to a 5 Log mL^{-1} reduction of *Escherichia coli* in an air flow after 10 sec of exposure to plasma.
Other researchers, **Dobrynin *et al.* (2010)** subjected *Bacillus cereus* and *Bacillus anthracis* to a non-thermal plasma treatment, observing 5 Log mL^{-1} inactivation of these strains after a 1 min treatment. **Moissan et al. (2011)** made theoretical references about the action mechanisms of microorganism-killing non-thermal plasma. They postulated that non-thermal plasma may cause damage to the membrane and cell wall, with consequent damage to DNA caused by UV light and the free radicals generated by the interaction of plasma with ambient air at the time of exposition. **Dobrynin *et al.* (2011)** demonstrated that there was a transport of charged molecules into the cell, such as hydrogen peroxide ($OH^-(H_2O)n$ and $H_3O^+(H_2O)n$) and that the efficiency of these radicals to promote microorganism death is much greater than free ions, such as O_3, N_3 and argon ions.
Although microorganism decontamination caused by NTP exists There are studies that suppose the mechanism of destruction of the cell. For this reason, the aim of this study was to evaluate the effect of non-thermal plasma on the viability of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 51811 and

Saccharomyces cerevisiae and, furthermore to determine the mode of action of plasma on these organisms, analyzing cell wall damage and DNA denaturation.

MATERIAL AND METHODS

Microorganisms

The strains of *Escherichia coli* ATCC 25922 (FDA strain Seattle 1946, American Type Culture Collection, E.U), *Staphylococcus aureus* ATCC 51811 (BioCyc Database Collection, Menlo Park, California. E.U), and *Saccharomyces cerevisiae* CTSA (a strain isolated from must of tequila and characterized for genus and species by molecular biology), were lyophilized and kept at -80 °C for preservation.

Dielectric barrier discharge reactor

The system made up by the radio frequency generator (RF) and the dielectric barrier discharge reactor (RDBD) was designed and built in the Laboratory of Plasma Physics from the National Institute of Nuclear Research, Mexico. The reactor consists of a pair of horizontal stainless steel circular parallel electrodes, 80 mm in diameter and 2 to 5 mm spacing regulated. The electrode below is fixed to the lower lid of the reactor by a shaft, while the upper one, in order to fix the gap, can be adjusted by means of a precision screw coupled to a similar shaft attached to the upper lid, the last is constituted by a tubular shaft allowing gas admission. The entire structure is insulated by means of a Pyrex® 200 mm long cylindrical structure with a 120 mm internal diameter (see figure 1). A Petri dish, containing a bacterial colony, is placed between both electrodes, acting as a dielectric barrier. The specially designed 13.56 MHz RF generator directly supplies the reactor parallel plates, applying a 30W input power and an output voltage of 850 volts with He-air mixture 1.5 LPM flow. The power in the system can be adjusted with a resolution in the order of milliwatts, as it has been designed to conduct studies on the characterization and interactions of active species and UV radiation from discharges with the microorganisms.

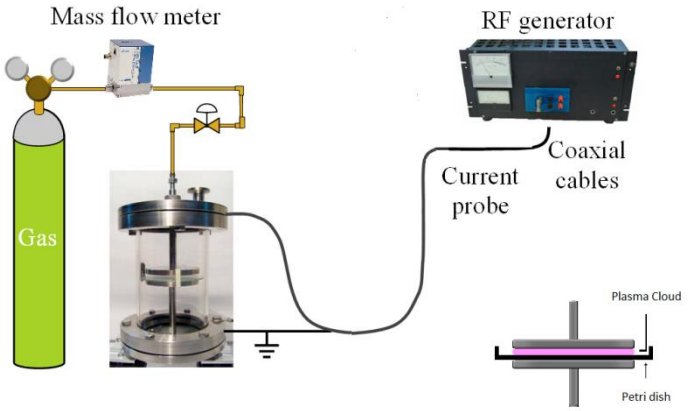

Figure 1 Experimental setup diagram implemented for the generation of plasma discharges

Microorganism culture

Prior to treatment with plasma, growth was achieved in the bacteria *E. coli* ATCC 25922 and *S. aureus* ATCC 51811 in nutrient broth (BD Bioxon) at 35°C for 24 h (MAI TERLAB Model 60, HINOTEK, China), and for *S. cerevisiae* yeast cultivation, liquid medium YPD was used (yeast extract and casein peptone 5 g/L dextrose 20 g/L) incubated at 30°C for 24 h. Subsequently, the cultures were centrifuged (Hermlez 300K, LabSource, France) at 3500 rpm for 5 min. The supernatant was removed and the biomasses washed with 1 mL saline, then centrifuged again; subsequently, three washes were performed with consecutive centrifugations at 3500 rpm for 5 min. Absorbance was then measured by spectrophotometer at 625 nm (Genesis 20, Thermo Spectronic, USA) and the number of cells was estimated to 10^9 CFU mL^{-1}according to the previously constructed 0.5 McFarland curve for conducting plasma treatment.

Microorganism exposure to NTP

On 1 cm^2 sterile filter paper (Whatman No.1), 20 µL of just one cell culture of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 51811 and *Saccharomyces cerevisiae* were placed at concentrations of 10^9 $UFCmL^{-1}$ according to the 0.5 McFarland scale. All samples, excepting one negative control, were subjected to non-thermal plasma exposure for 10, 20, 40, 60, 80, 100, and 120 s. After that, samples were placed in sterile tubes containing 9.9 ml 0.1% peptone water, stirred a few minutes on a Vortex (Vortex-Genie 2 mixers, Scientific Industries, Inc. USA) then decimal dilutions were made. Reseeding for each sample was promptly carried out with the plaque emptying method, using 1 ml of the dilution. For *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 51811, nutrient agar was used, incubating the petri dishes at 35 °C for 24 to 48 h; for *Saccharomyces cerevisiae* CTSA, potato dextrose agar (PDA) was used and incubated at 28°C for 24 to 48 h; finally, colony counting was performed (CRAFT ® colony counter, Interscience, France). The mean was calculated and expressed as $CFUmL^{-1}$ (colony-forming unit per ml sample).

Qualitative assessment of damage to the wall of the cell membrane.

The technique described by **Klotz *et al.* (2010)** was employed to assess the damage to the membrane and cell wall of the bacteria *Staphylococcus aureus* ATCC 51811, *Escherichia coli* ATCC 25922 and *Saccharomyces cerevisiae* CTSA. This technique is based on interaction with propidium iodide double-stranded DNA. Samples of cell suspensions of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* in phosphate buffer saline solution were prepared at an 0.2 OD_{680} (spectrophotometer model Genesis 20, Thermo Spectronic, USA) corresponding to a concentration of ~ $2.4x10^2$ $CFUmL^{-1}$, this population density providing the optimum resolution for cell observation in the microscope. Twenty µL of the suspension of each strain were placed on sterile glass slides, and then the slides were exposed to plasma for 10, 20, 40, 60, 80, 100 and 120 s. Subsequently, 5µL propidium iodide were added (MP Biomedicals, USA) at a concentration of 1 mg/mL. Then, 10 µL methylene blue dye was added (EMB Bioxon) at a concentration of 15 mg/mL (adding methylene blue was performed in order to neutralize the fluorescence which originates by contact with propidium iodide cells walls of yeasts, giving false positive reading) and dried for 5 minutes. All treated samples were incubated in the dark (22 ± 1°C/20 min) and microbial cells were observed with the aid of a fluorescence microscope (Olympus CKX41, USA) at 480 nm using 40X magnifying lens. From observations, an increase of cell fluorescence was noticed, indicating microorganism membrane or cell wall rupture.

DNA extraction and analysis by agarose gel electrophoresis

For assessing DNA damage, 100 µL microorganism cells (~ $2.4x10^2$ $CFUmL^{-1}$) were placed on a sterile glass slide. Each set of seven slides were exposed to plasma for 10, 20, 40, 60, 80, 100, and 120 s. After treatment a DNA extraction was carried out on *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 51811 using the genomic DNA extraction kit (Promega). The total DNA of *Saccharomyces cerevisiae* CTSA was carried out with the Rapid Yeast Genomic DNA Extraction Kit BS8227-50P (Bio Basic Canada Inc). In order to analyze DNA fractions, a gel of 0.7% agarose plus 1X SYBR safe (Invitrogen) in TAE buffer (Tris/acetate/EDTA), was charged with the same amount of DNA extracts (2 µg) and subjected to electrophoresis for 1h at 75 V. Gel image was documented with a EZ Gel Doc (Bio Rad, USA). As positive control, a sample of DNA treated with a restriction enzyme (*Eco*RI) was included in the gel previously described.

Statistical analysis

The present study was conducted 3 independent trails (replicates). Statistical analysis was performed using Statgraphics Centurion XV V15.2.06 (StatPoint, Inc., USA). The microbiological assessment data were subjected to an analysis of variance (ANOVA), and the mean values were compared using Fisher's least significant difference (LSD) at a level of 0.05.

RESULTS AND DISCUSSION

Death kinetics in microorganisms and decimal reduction value,

The results of microbial death kinetics are shown in Figures 2a, 2b and 2c (survival curves). When comparing the inactivation kinetics of plasma, differences in microbial reduction levels were found. A critical parameter for evaluating the efficiency of a microbial reduction technology is the decimal reduction D value (**Klotz 2010; Pflug 2000**). This value is a time required to inactivate 90% of the microbial population, and was calculated taking the negative inverse of the slope from the linear equation for each phase of microbial death, as expressed in the following equation:

$$D = 1/m$$

where:

D is the time (in minutes) required to inactivate 90% of the microbial population and ***m*** is the slope of the linear equation for each phase of microbial death

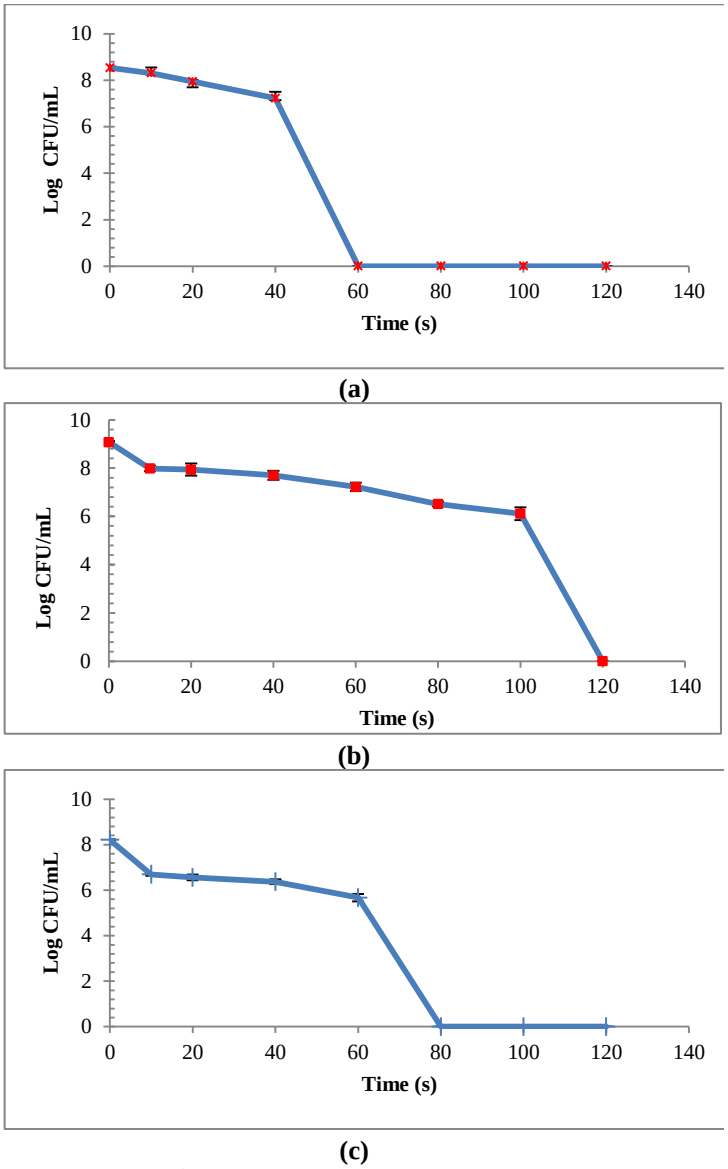

Figure 2 Kinetics of microorganism death when exposed to non-thermal plasma (850 V) for 120 s. **a)** *Escherichia coli* ATCC 25922, **b)** *Staphylococcus aureus* ATCC 51811, **c)** *Saccharomyces cerevisiae* CTSA.

After exposure of different microorganism strain to plasma, it was observed that inactivation kinetics of *Escherichia coli* ATCC 25922 (Figure 2a) showed a biphasic behavior ($p<0.05$) where, two calculating the decimal reduction in each of the slopes of the biphasic function, it can be observed a significant reduction (Table 1). We observed following 60 s of plasma exposure, a rapid reduction of microbial population, where *E.coli* was inactivated. In the case of *Staphylococcus aureus* ATCC 51811 (Figure 2b), microbial inactivation was observed from 120 s of cold plasma treatment. Three phases of microbial reduction were observed with significant differences ($p<0.05$) with logarithmic jumps 9.2, 45.2 and 3.4 seconds respectively for each slope. As in *E. coli*, *S. aureus* show a total elimination of microbial load after 2 minutes, initial concentration being reduced Log 9.07 ± 0.18 CFU ml^{-1} to <Log 0.001 CFU ml^{-1}.On exposure to NTP of the yeast *Saccharomyces cerevisiae* CTSA also presented three slopes microbial reduction with statistically significant differences ($p <0.05$) (Table 1).

Table 1 D Values (decimal reduction) in microorganisms exposed to cold plasma at 850 Volts

D Values [1]	Microorganisms		
	Escherichia coli ATCC 25922	*Staphylococcus aureus* ATCC 51811	*Saccharomyces cerevisiae* CTSA
D1	$30.08^{a, §}$±8.31	$9.22^{a, †}$±1.23	$6.56^{a, †}$ ±0.19
D2	$2,79^{b, §}$ ±0.10	$45.26^{b, †}$±1.64	$51.02^{b, †}$±7,06
D3	-------	$3.54^{c, †}$±0.56	$3,54^{c, †}$±0.10

[1]D values (in seconds) correspond to the average ± SD, n = 3.
The letters a, b, c, represent statistical differences of D values within each microorganism.
Symbols § and † represent statistical differences between D values compared between different microorganisms.

The significant reduction in yeast concentration occurred in the time 60 to 80 s of treatment with a D = 51.02 seconds (Figure 2C); after 80 s treatment, a reduction of up to 8.21 ± 0.18 <0.001 log CFU ml^{-1} was obtained. *S. aureus* was more resistant to NTP treatment with 120 seconds for complete removal. Compared with *E. coli* being 50% higher and for *S. cerevisiae* 33.3%, respectively (Figures 2a, b, c). The reducing the concentration of microorganisms is evident after 60 s of treatment. The obtained time of inactivation and D are different and these results are genus and species dependent. For instance, the microbial inactivation kinetics of *S. aureus* and *S. cerevisiae* showed three inactivation phases and three D values, while *E. coli* had only two death phases. The fact that *S. aureus* had a higher D value than did *E. coli* suggests that peptidoglycans function as a physical shield against plasma. This was also observed with *S. cerevisiae*, the cell wall structures of which differ from those of bacteria and are more difficult to rupture mechanically. Thus, the reactive species produced by NTP interaction with ambient air, have greater difficulty breaking the cell wall or membrane of Gram-positive bacteria and yeasts than of Gram-negative bacteria. According to the results of the death kinetics of the three microorganisms, we think that the first microorganism death phase could be caused by the formation of free radicals, which interact on the components of cell walls, while in the second phase; the action of free radicals would be of greater intensity as it would be on weakened cell walls. **Laroussi (2002)** describes that during microbial death kinetics, several phases occur and explain that the first phase (D1) is produced by the action of UV light, the second phase (D2) is attributed to a process erosion by the active species (free radicals), and in the third phase (D3) the UV light acts by damaging the genetic material of microorganisms. Nevertheless, **Georgescu *et al.*(2010)** details the inactivation of microorganisms because of NTP can be due to the charged particles, electric fields, UV photons and some reactive species such as atomic oxygen, metastable oxygen molecules, ozone and hydroxyl radicals, which are commonly found in a gas discharge. In particular, when helium is used as the main gas and starts to be in contact with air some of the generated species are oxygen atoms, OH radicals, nitrogen atoms, NO radicals, nitrogen ions, etc.

We could associate also this phenomenon to peroxidation lipid and polysaccharide which cause bacterial inactivation (**Dobrynin et al. 2009**). We believe that the difference between the compositions of the cell walls of these microorganisms is the cause of the different inactivation behaviors among *E. coli*, *S. aureus* and *S. cerevisiae*, the damage of NTP may depend on the thickness of the cell wall. For example *E.coli* (Gram-negative) present a thin layer of peptidoglycan, located between two layers of phospholipids, which are attached to an outer membrane through a lipoprotein, whereas Gram-positive bacteria such as *Staphylococcus aureus* have a thick layer of peptidoglycan, teichoic acids and two kinds of lipoteichoic acid (**Kubista, 2006; Tortora, 2012**). On the other hand, in yeast, three main groups of polysaccharides form their cell wall: 40% mannoproteins, 60% β-glucan and 2-5 % of chitin (**Klis *et al.* 2006**).

Damage to the cell wall

The damage of the cell wall is determined qualitatively, where the results of *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 51811 and *Saccharomyces cerevisiae* were treated with NTP and are shown in Figure 3. From 10 and 20 s, it began to observe an orange colored and stronger fluorescence was obtained at 60 s of treatment for all microorganisms. It is evident that with increasing time of treatment with NTP causes the breakdown of the cell wall in bacteria and yeast for the release for internal cellular material where there are DNA in the case of prokaryotic and eukaryotic. The propidium iodide have contact with the freed genetic material of the microorganism causing the fluorescence and depending of the genetic concentration material of its intensity (Figure 3).

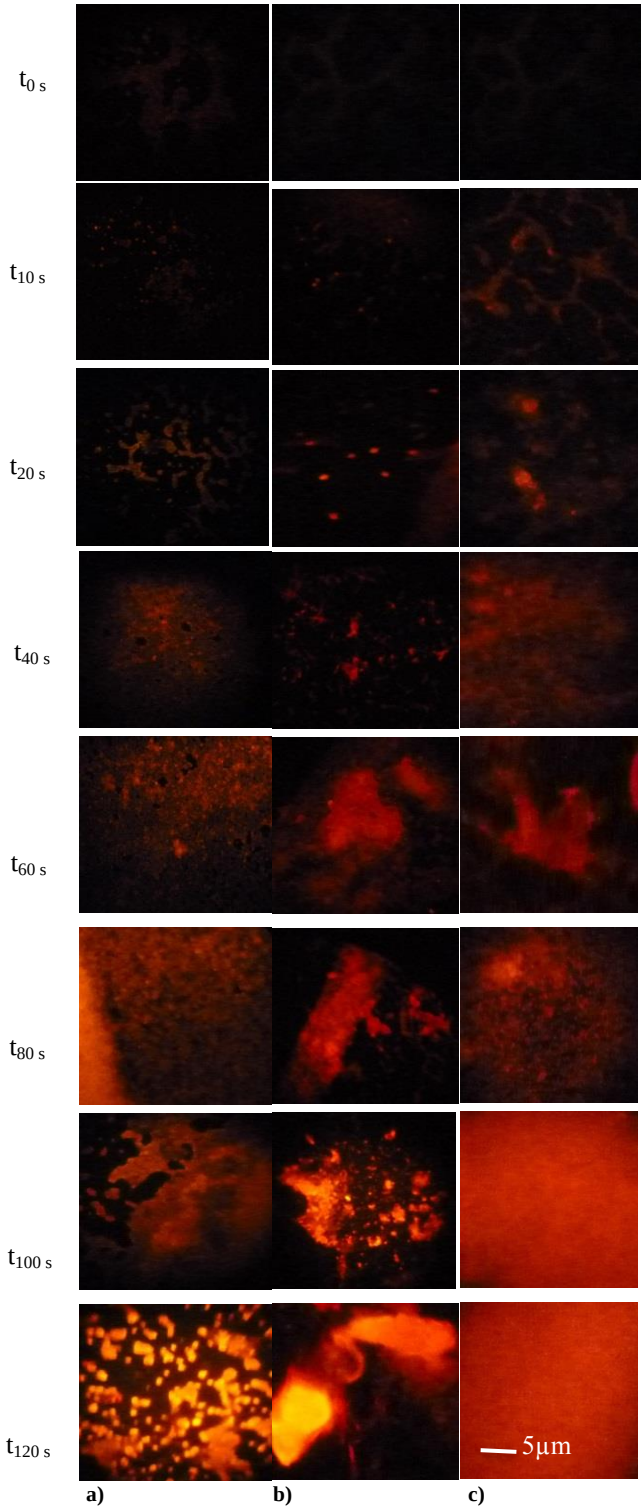

Figure 3 Propidium iodide fluorescence (1mg/ml). a) *Escherichia coli* ATCC 25922, b) *Staphylococcus aureus* ATCC 51817 and c) *Saccharomyces cerevisiae* CTSA, exposed to non-thermal plasma at different times. Degree of magnification (400X).

It can be noted, that cell walls suffered lysis with increased plasma treatment time (Figure 3). We assumed that the increase in fluorescence was directly related with the interaction of propidium iodide with the yeast nucleic acids after lysis. It has been shown that in the case of bacteria, the combined effect of the magnetic field and free radicals provoke the electroporation phenomenon (**Azharonok, 2009; Dobrynin, 2011**), breaking the cell walls and causing the release of genetic material with a probable degradation of DNA. *E. coli* ATCC 25922, *S. cerevisiae* CTSA and *S. aureus* ATCC 51811 treated with NTP show an increase in fluorescence intensity in function of treatment time after 10, 20 and 40 s respectively, indicating that there is an increase in the interaction of DNA with interact propidium iodide, (**Van Frankenhuyzen, 2011**). This would indicate that as treatment times of treatment in different microorganisms, causes an increase of lysis in the cell wall that leads them to death. However, **Gadri *et al.* (2000)**, reported that even the similar D value of Gram-positive and Gram-negative exposed to non-thermal plasma, in Gram-negative bacteria lysis and fragmentation occurred in the cell wall, but in Gram-positive bacteria this did not happen.

Microorganism DNA analysis in agarose gel

The technique of agarose gel electrophoresis was implemented with the aim of evaluate the effect on the DNA after the application of NTP at different times. Figure 4 shows the agarose gel electrophoresis of DNA obtained from the three different microorganisms, showing the integrity of DNA, interpreting the results as previously done by **Ryu*et al.*(2013)** for a treatment with NTP of *S. cerevisiae* AH109. Regarding *Escherichia coli* ATCC 25922, at 60 s we observed that treatment with NTP (lanes 1 to 5) do not cause an observable damage to the DNA. However, after 80 s treatment (lane 6) the DNA band which represents the intact DNA disappears. Similarly, the integrity of the DNA of *S. aureus* ATCC 51811 and *S. cerevisiae*s showed signs of degradation until 120 s of treatment with NTP, in both cases it could be observable the presence of a DNA as an intact band.

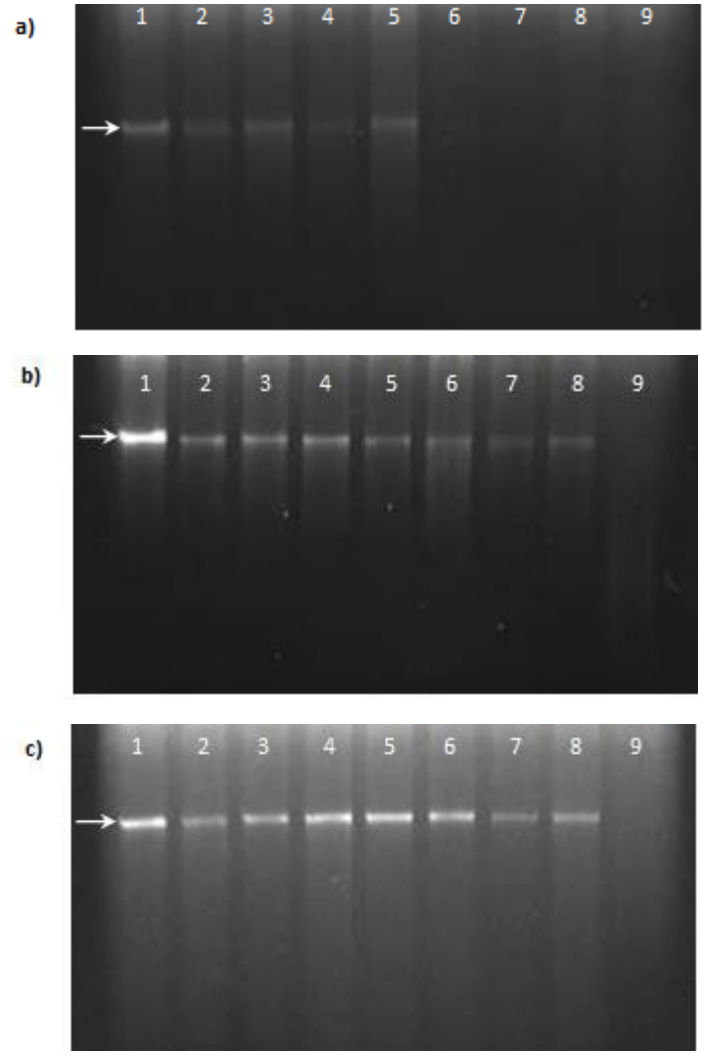

Figure 4 Kinetics of degradation DNA in microorganisms exposed to non-thermal generated with helium gas. **a)** *Escherichia coli* ATCC 25922, **b)** *Staphylococcus aureus* ATCC 51811, **c)** *Saccharomyces cerevisiae* CTSA. Lane 1) 0 s, 2) 10 s, 3) 20 s, 4) 40 s, 5) 60 s, 6) 80 s, 7) 100 s, 8) 120 s 9) DNA control (denatured DNA with enzyme restriction EcoRI). Terms of run: 0.7% agarose, 75 volts and 37 mA. Run time: 1h. Note: The horizontal arrows indicate DNA bands of the microorganisms treated with non-thermal plasma.

In the case of DNA degradation, *Escherichia coli* ATCC 25922 was the microorganism that suffered more fragmentation of DNA at a lower time (60 s) on (Figure 4a); however, for *S. aureus* ATCC 51811 and *S. cerevisiae* CTSA, there was not DNA fragmentation observed after of 120 s of treatment (Figures 4b, c). According to **Tortora *et al.* (2012),** the cell wall of Gram-positive bacteria contains a thicker layer of peptidoglycan with respect to Gram-negative bacteria. Since they are composed of a peptidoglycan layer, the cell wall of *E. coli* ATCC 25922 (Gram-negative) probably suffered lysis in less time than *S. aureus* ATCC 51811 (Figures 3a, b), and as a consequence, *E. coli* ATCC 25922 DNA

fragmentation was higher than that of *S. aureus* ATCC 51811 (Figures 4a and b), and as a consequence, *E. coli* ATCC 25922 DNA fragmentation was faster than the *S. aureus* ATCC 51811. Another important aspect in the case of prokaryotic bacteria is that DNA is in free form in the cytoplasm (**Kubista, 2006**), a feature that favors DNA interaction with NTP constituents and its interaction with ambient air, such as free radicals, UV light, charged particles (**Pflug, 2000**). Evidence exists that low-energy electrons cause damage to DNA (**Gadri, 2000**); additionally, UV light causes photodimerization in adenine (**Alizadeh, 2011**).

The DNA of *Saccharomyces cerevisiae* CTSA does not show any fragmentation (Figure 4c) demonstrating that *S. cerevisiae* CTSA cell wall consists of glucans, mannoproteins, and chitin, making it more resistant to cell wall rupture than the case of Gram-negative bacteria (*E. coli* ATCC 25922), as is noted in the increase of fluorescence (the experiments with the propidium iodide technique). However, *S. aureus* ATCC 51811 was more resistant than *S. cerevisiae* CTSA, perhaps because of its thick peptidoglycan layer. Another aspect about the yeast *S. cerevisiae* CTSA is that it is a eukaryote and has a membrane that protects its nuclear DNA against free radicals, UV light and charged particles generated by NTP. It probably obstructs plasma in penetrating to the core and destroying DNA in yeast, while prokaryotes (bacteria) do not have this membrane (**Kubista, 2006**).

Rastogi *et al.* (2010) submitted viral DNA samples extracted from *E. coli* cells and bovine serum albumin (BSA) to ultraviolet radiation exposition. The results showed that viral DNA suffered degradation from 10 s on, and after 20 s of treatment, DNA was completely degraded. The research by **Morales-Ramírez *et al.* (2013)**, who submitted mouse leukocytes to exposure to RF non-thermal needle plasma at different distances (0.1 and 0.5 cm from the tip of needle to samples), obtained complete DNA fragmentation at 0.1 cm. However, the presence of proteins around DNA molecules can protect them from the effects of plasma generated reactive species (NO, HO*, O_3, O_2) and thereby reduce damage (**Morales-Ramirez, *et al.* 2013**).

Finally, **Dobrynin *et al.* (2009)** analyzed the interaction of floating electrode dielectric barrier discharge (FE-DBD) in living tissues and cells. They discussed and presented the biological action (reactant species ROS) and physical mechanisms in the tissues and cells. They proposed three major hypotheses for the results observed: **1)** ROS metabolism is different in prokaryotic (bacteria) and eukaryotic (mammalian) cells, e.g. while human cells have protection from O_2, bacteria either lack it completely or their resistance is lower. **2)** higher order organisms have developed more resistance mechanisms to external stress (osmotic pressure changes, ROS, chemical and biological poisons, etc.), in other words, bacteria usually act as a single cell while mammalian cells, especially those organized into tissues, communicate with each other possibly lowering the effects of the applied poison; finally, **3)** bacterial cells are usually much smaller than mammalian cells and thus have much higher surface to volume ratio, therefore simply a lower dose of poison is required to inactivate the same number of bacteria as opposed to mammalian cells. They stated that although their results are potentially promising, many unanswered questions and gaps in understanding remain and that selectivity is still an open question where deeper understanding is needed about the mechanism of interaction of plasma with bacterial and parasite cells.

CONCLUSION

The action of non-thermal plasma and its active components on microorganisms *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 51811 and *Saccharomyces cerevisiae* CTSA caused their cell death during exposure to NTP. This induces breakdown of the microorganism cell walls, despite differences in their constitution, in *E. coli* cause the release of the genetic material (DNA) and its subsequent degradation. However, *S.aureus*. ATCC 51811 and *Saccharomyces cerevisiae* CTSA the release of this material was not gotten out. With this study we have shown that microorganism cell wall is the main organelle on which the cold plasma energy acts. Therefore this death process mechanism consists of the action of reactant species or oxidants produced by the NTP which cause breakdown in the cell wall eventually leading to microorganism death.

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