Glow Discharge Plasma Sterilization of Prokaryotic Microorganism Through Electrostatic Plasma

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Abstract

Plasma is the fourth state of the matter. In this state the matter has enough energy to delocalize the subparticles of the atoms that make up the material. At the subatomic level plasma is a mixture of highly energized particles capable of causing destruction of DNA, mostly through free radicals, and disabling cell reproduction. Due to the harsh conditions that current sterilization processes require on the materials, their use has been frequently limited. The main objective of this research was to develop a sterilization method using air as ionized gas (air in plasma state) by the Glow Discharge method that would provide an a less aggressive alternative to current sterilization methods. The physical-chemical conditions of the "cold-plasma state" allows us to expose a vast range of materials to plasma, causing minimal alterations, if any, in their physical and chemical constitution. The results obtained experimentally suggest that the plasma sterilization method using air as gas is possible. When evaluating the correlation of the variables pressure, current, and time on the CFU variable, it was found that time is the most influential variable to reduce the formation of CFUs when exposed to plasma. Based on the results obtained, 6 minutes of exposure to air in its ionized state are sufficient to reduce the possibility of development of CFUs to almost 0%.

Introduction

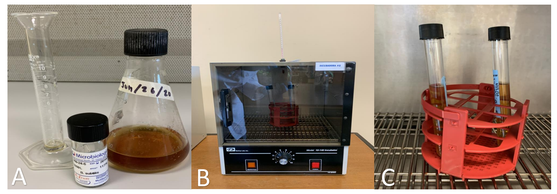
Effective sterilization processes have been one of the primary factors for the success and continued development of modern medicine. However, throughout history, numerous cases have been documented in which clinical infections were developed after surgical procedures, due to possible failures in the sterilization process of surgical equipment [3]. There are different types of sterilization methods. Traditional chemical and physical sterilization methods do their job, but they share a common limitation. The range of materials where they can be used is limited due to the tough conditions (highly corrosive, high temperatures or pressures, etc.) needed in order to meet the sterile conditions of an object [1][2]. The use of certain compounds or elements such as helium and water (He and H_2O) in the plasma state have a great potential to perform highly effective microbial sterilization processes at low temperatures, according to a study carried out at Yamanashi University, Japan in 2004 [5]. During the development of this study, we expected to learn the timeframes, possible compounds, frequency regimes, currents and voltages, necessary to develop an effective method of sterilization in the plasma state. With equipment capable of operating at low temperatures without the need of highly reactive chemicals, sterilization conditions would allow the method to be applicable to almost any material.

Objectives

This project is a continuation of the research carried out by Morales and González in 2019. Based on the data of that 2019 research, some inconsistencies were found. Our goals where divided in three stages: Stage one of our research was focused on finding the factors that produced the inconsistencies in the results obtained by Morales and Gonzalez. Once the possible factors that may have caused these inconsistencies were identified, several suggestions were made to the methodology. In stage two, changes suggested to the methodology were put to test. Stage two findings were positive and consistent. Stage three aimed to develop time and effectiveness curves with different variables that may be involved in the sterilization process. By doing so, it is possible to establish patterns of behavior for bacterial exposure in the plasma environment in relation to time. The main goal of this project is to develop a sterilization method using air as ionized gas (plasma) and based on that, develop a device. This device should sterilize a wide range of materials in a short period of time at low cost.

Materials & Methods

Activation of *Bacillus subtilis*



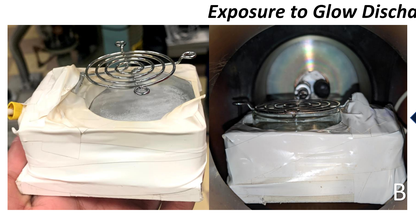
[Fig 1 (A, B & C)] Activation process of *B. subtilis* bacteria in Tryptic Soy Broth (TSB). This medium is left in the incubator for 24 hours at a temperature of 37° C.

Preparation and Cultivation of Bacillus subtilis Petri Dishes

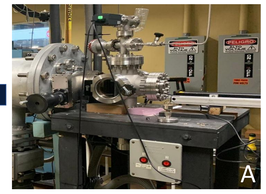
[Fig 2 (A & B)] 25 minutes of sterilization process in the Autoclave for the Petri dishes following the preparation of Tryptic Soy Agar (TSA) in a 125mL Erlenmeyer flask where 5g of TSA are used for 21 Petri dishes.

[Fig 3 (C)] Preparation of the Petri dishes where a total of 6mL of TSA is added.

Exposure to Glow Discharge Plasma

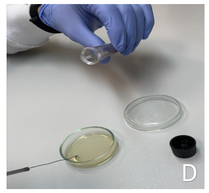


[Fig 7 (B)] Aluminum and Teflon bases. Petri dishes are placed here to go through Glow Discharge plasma exposure. The time periods were from 15, 13, 11 minutes, and then from 10 minutes to 1 minute reducing by 1 minute each period. Each time period consist of 6 samples making it one time-group-sample.



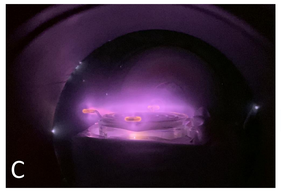
[Fig 6 (A)] Plasma chamber through Glow Discharge and Electron Cyclotron Resonance Heating (ECRH) at Plasma laboratory at the PUPR - machine where bacteria sterilization samples were placed.

[Fig 5 (E)] Sterile bags for transportion of biological material. Used to transport bacteria from laboratories to avoid external contamination of samples.



[Fig 4 (D)] The inoculation of *B. subtilis* were performed using a 10μm needle loop in the biological cabin.

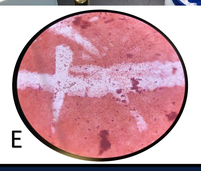
Exposure to Glow Discharge Plasma



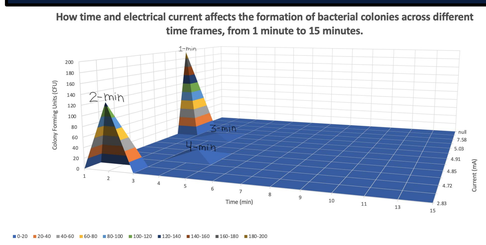
[Fig 8 (C)] Showing direct Glow Discharge through the Petri dish.

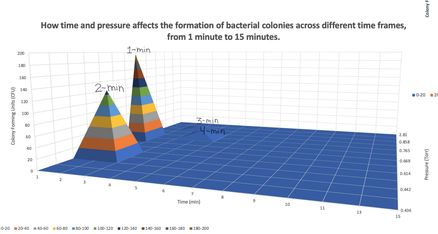
[Fig 9 (A & B)] For each time-group sample, one no-plasma exposed Petri dishes were inoculated with active bacteria into a fresh agar medium. This control sample confirm that active *B. subtilis* were presented in all plasma exposed Petri dish samples. All No-plasma Petri dishes (NPPd) are placed into the incubator along with those that were exposed to Plasma to to compare the effectiveness of the sterilization.

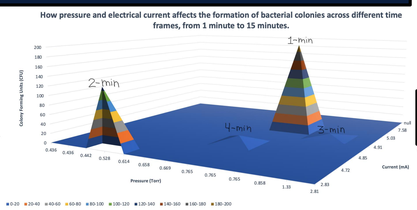
[Fig 10 (C, D & E)] After 24-hour of incubation period, samples are analyzed through a microscope to determine if there is microbial growth. If any CFU is found, they are analyzed to determine if in fact they are *B. subtilis* by its CFU shape. If they cannot be identified, they undergo through a simple staining and smear process where it is determined if the bacteria is Gram positive or Gram negative. *B. subtilis* is a Gram negative bacterial species.

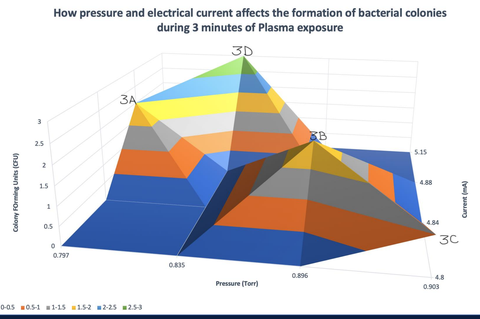


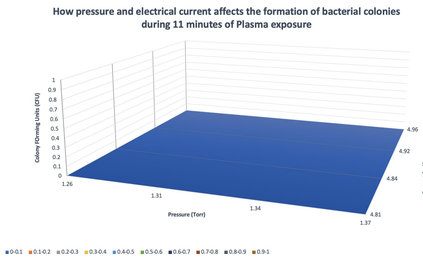
Data











Analysis & Results

Three dimensional graphs were made by plotting the values of the variables colony formation units (CFU), time, and the average values of each time-group sample for the variables pressure, electric current. To facilitate understanding of the relationship between variables, 3D graphs were made to provide a spatial perspective of the possible association between variables. When evaluating the CFU graph in relation to variables pressure and current, two significant peaks are seen, one at a pressure of 0.528 Torr and 2.83mA, and the other at 0.765 Torr and 5.03 mA. These peaks correspond to the 1-minute and 2-minute exposure times. Plotted values appear to indicate that pressure has a greater effect on bacterial growth than electrical current, given that there is increased density of CFU in the regions of high pressure and higher electric current. In other words, these results suggest that low pressures reduce the possibility of CFUs. The pressure vs time graph suggests, again, that pressure has a negative effect on CFU densities when exposed to Plasma. Lower pressures reduce the growth of bacteria. However, the time variable seems to be much more influential in the possible formation of colonies as the time vs current graph shows a similar but stronger decrease in the CFU density. The longer time of exposure to plasma, the greater the reduction on bacterial growth. With time being a stronger factor than pressure. On the other hand, the current variable does not seem to be very relevant, since the CFU values do not differ much between the different current regions. As a reference, the graph that correlates the variables pressure, current, and CFU for 3 minutes, is related to the global results where low pressures and high current values reduce the possibility of CFUs. On the other hand, the graph of plasma exposure for 11 minutes shows a completely flat surface, even under high-pressure and average currents values. This suggests that the time factor is the most influential variable in relation to the CFU variable.

Conclusion

Based on the results obtained, the use of air as ionized gas (plasma) as a method of sterilization that might be applicable to a wide range of material seems promising. After evaluating the correlations of the variables discussed, the variable time under plasma exposure seems to be the most influential factor for the expression of the variable CFU. Based on the results obtained, 6 minutes should be sufficient to reduce the possibility of bacterial growth to almost 0%.

Future Work & Recommendations

Future works could be focused on the addition of a pressure regulator valve to the Glow Discharge plasma chamber to keep constant pressure during plasma exposure. The addition of a multimeter that allows a more precise measurement of the current values should be also considered.

Acknowledgements

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