Glow Discharge Plasma sterilization as an alternative for ethylene oxide sterilization (EtO)

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Abstract

This work is considering plasma sterilization as an alternative for ethylene oxide sterilization (EtO). Ethylene oxide is a carcinogen that represents a threat to the environment and human health. During the COVID-19 pandemic, there was a shortage of sterilized medical equipment due to the dependence of the United States in EtO and the lack of alternatives for this sterilization method. In this study, the student sought to replicate the methodology applied by previous researchers of the Undergraduate Research Program for Honor Students (URP-HS) Omar Cepeda and José Martes (2020). The results that were obtained show that the plasma was partially effective at sterilizing *Bacillus subtilis* vegetative cells. Results also demonstrate that the pressure is more influential in the success of sterilization than the time of exposure. Therefore, the application of a pressure valve is necessary to improve the regulation of the pressure inside the plasma chamber and have more control of the sterilization procedure. The researcher recommends performing plasma diagnostics to obtain the temperature and density of the plasma and relate them to the effectiveness of sterilization.

Introduction

Ethylene oxide (EtO) as a sterilization method:

- EtO is effective at eradicating microorganisms without damaging the function and composition of medical devices.
- Due to its toxicity, many EtO facilities were closed before the pandemic.
- During the COVID-19 emergency, there was a serious shortage of this gas, which led to the lack of sterile medical equipment.
- Many EtO sterilization facilities were authorized to reopen.
- Increased use of this toxic carcinogen.

Glow discharge plasma as an alternative:

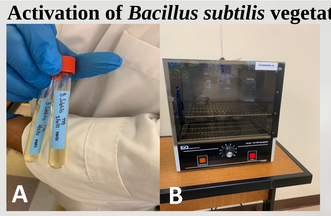
- Plasma is defined as the fourth state of matter, and it is composed of a mixture of ions, protons, and electrons.
- Plasma is effective in sterilizing medical equipment without exposing them to harsh conditions that affect their functionality and composition.
- Basic sterilization mechanisms:
  - Damage to the DNA caused by plasma UV irradiation (Moisan et al., 2002).
  - The erosion of the microorganism through etching (Moisan et al., 2002).
  - Erosion of the microorganism through intrinsic photodesorption (Moisan et al., 2002).

Objectives

- Replicate the sterilization procedure performed by Martes and Cepeda in 2020 for a range of 5 to 15 minutes.
- Study the effect of Glow discharge plasma in resistant bacterial endospores.

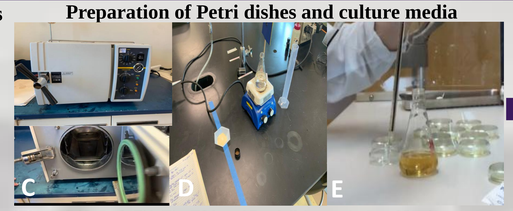
Experimental Methods

Activation of *Bacillus subtilis* vegetative cells



[Figure 1 (A & B)]: Activation of *B. subtilis* bacteria in Tryptic Soy Broth (TSB) is presented. The culture is left to incubate for 24 hours at a temperature of 37°C.

Preparation of Petri dishes and culture media



[Figure 2 (C, D, & E)]: The TSA and Petri dishes undergo a 25-minutes sterilization cycle in the autoclave. Each Petri dish contains 6 mL of TSA.

Inoculation of samples

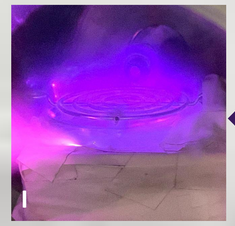
[Figure 3 (F)]: Inoculation of experimental samples using a sterile inoculation loop.

Packaging of samples

[Figure 4 (G)]: Each sample was separately packaged inside a "Whirl-Pak" bag and transported inside a biohazard bag.

Exposure to Glow Discharge Plasma

[Figure 5 (H)]: Plasma chamber where experimental samples were sterilized inside an aluminum and Teflon base. The rack that goes over the Petri dish ensures the formation of the plasma cloud over the surface of the agar. Each sample was exposed to the plasma in a range of 5-15 minutes.

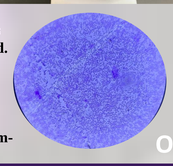


[Figure 6 (I)]: Glow discharge cloud formed over the entire surface of the Petri dish.

Retrieval and analysis of results

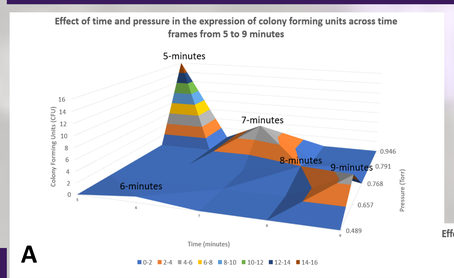
[Figure 7 (J)]: Non-plasma Petri dishes (NPPd) were also inoculated and directly placed in the incubator with the sterilized group.

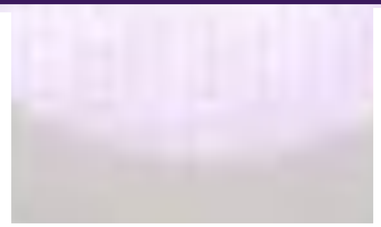
[Figure 8 (K)]: After a 24-hour incubation, experimental samples are observed to analyze the morphology of the CFUs. If the bacteria cannot be identified, smears are prepared, and the Gram staining technique is performed.

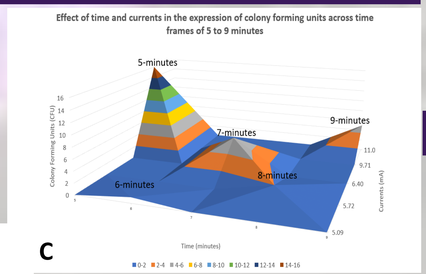


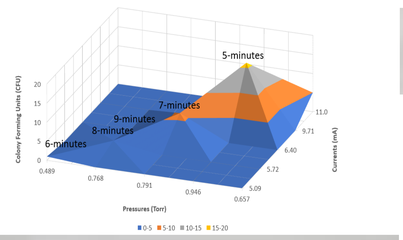
[Figure 9 (L, M, & O)]: Smears are prepared by heat fixing the samples The prepared slides are then stained. Once samples are dry, they are observed under the microscope to determine if the bacteria present is Gram-positive (purple) or Gram-negative (pink). *B. subtilis* is a Gram-positive bacteria.

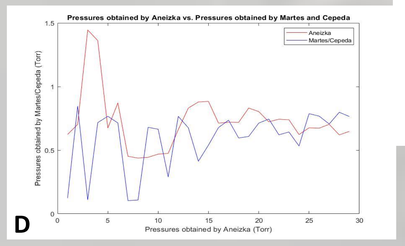
Collected Data

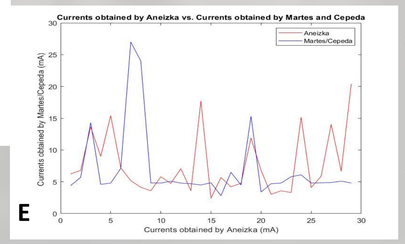












Analysis and Results

- Sterilization of *B. subtilis* vegetative cells was partially achieved.
- Compared to the work of Cepeda and Martes (2020), time was not the most determining factor in the presence of CFU.
- The presence of CFU depends on both pressure and current.
- The parameter of current depends on the pressure because the current travels through the mass inside the plasma chamber.
- A lower density of CFU was observed at lower currents and pressures.
- At greater times of exposure, there is a lower density of CFUs.
- It seems that pressure has a greater influence in the presence of CFU than time.
- Pressures were inconstant because the present equipment does not enable a better regulation.
- The inconsistency in pressures led to a discrepancy in the obtained currents.

Conclusions

- The obtained results show that Glow discharge plasma was partially effective at sterilizing *Bacillus subtilis* vegetative cells.
- A mechanism to improve the regulation of the pressure inside the chamber is necessary to have better control of the sterilization.
- It was not possible to perform the sterilization of endospores due to several inconveniences.

Future work and recommendations

- Perform plasma diagnostics to obtain temperature and density of the plasma and relate them to the effectiveness of sterilization.
- Implement a pressure valve to better regulate the pressure inside the chamber.
- Applying an API phenotypic test for more accurate identification of the bacteria present after sterilization.

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