Air-Based Plasma and its Impact on Bacillus subtilis: Implications for Healthcare Sterilization

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Undergraduate Research Program/HS – PUPR

2023-2024

Abstract

This study addresses the limitations of current sterilization methods in the healthcare industry, which expose operators to carcinogens and reproductive hazards, the environment to pollution, and compromise the integrity of polymer-based medical devices. This research focused on generating plasma using a custom-designed electrode system with aluminum and stainless steel as conductive materials and 3D-printed polylactic acid (PLA) as insulation. The electrode system was designed to operate at Normal Glow Discharge conditions, achieving a pressure of 1.5×10^{-1} Torr, a voltage of 500 V, and an average current of 2.21 mA. The experiments revealed that TSA solution volumes significantly affect vacuum performance and plasma generation, highlighting the need for optimization to improve efficiency and effectiveness.

Introduction

Current sterilization methods in the healthcare industry can negatively impact equipment integrity, human health, and the environment (Rutala, 2008). Plasma, a promising alternative, is created by applying high voltage across electrodes to ionize gas, forming an electric field that generates plasma (Nwabor, 2022). Effective plasma generation relies on proper insulation, voltage control, electrode materials, and gas pressure. In this study, Tryptic Soy Agar (TSA) was used to grow *Bacillus subtilis* for testing sterilization efficacy. TSA, which includes casein peptone, sodium chloride, soy peptone, and agar, was sourced from MP Biomedicals. However, TSA's performance was compromised under vacuum conditions. To address this, various TSA volumes and solidification methods were tested to minimize vacuum impact to the solution.

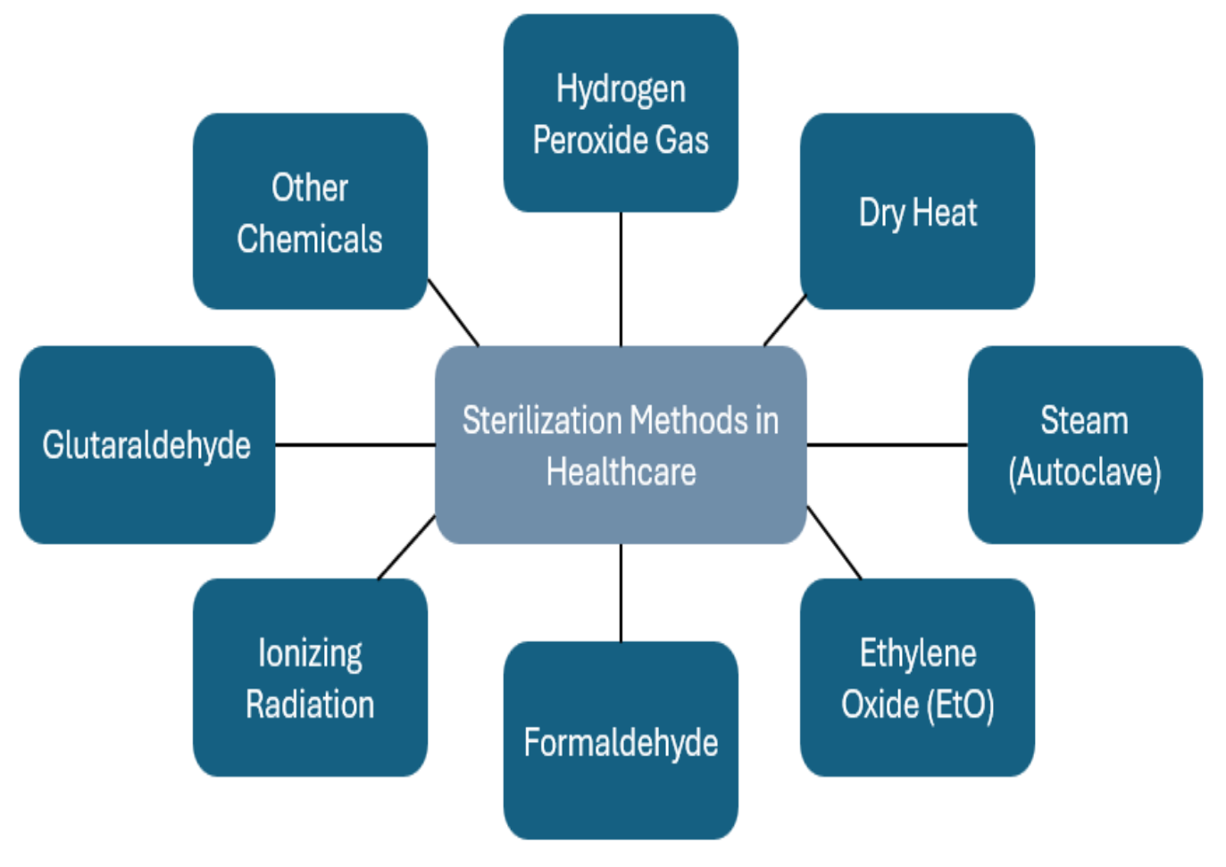


Figure 1. Sterilization Methods in Healthcare

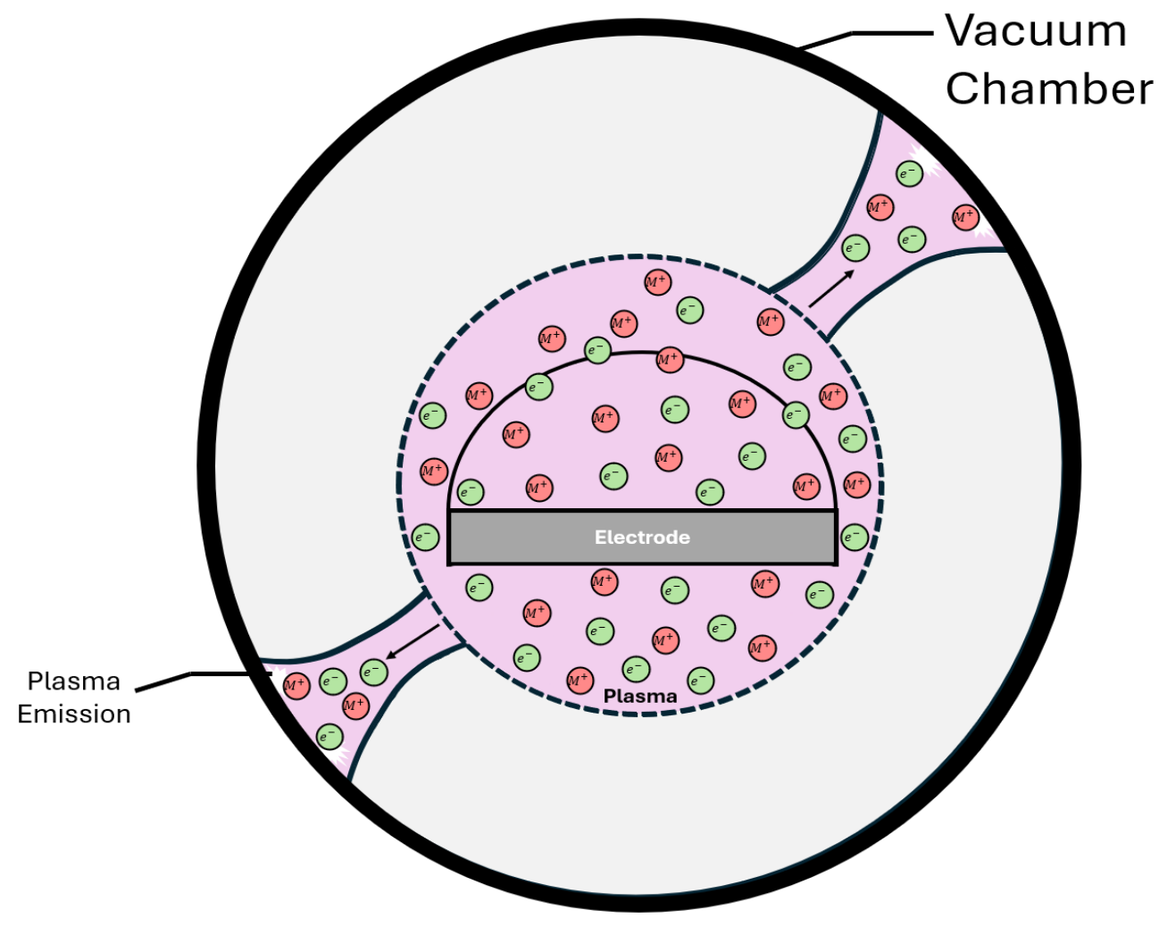


Figure 2. Electrostatic Confinement of Electrode

Figure 3. Plated Tryptic Soy Agar (TSA) from MP Biomedicals

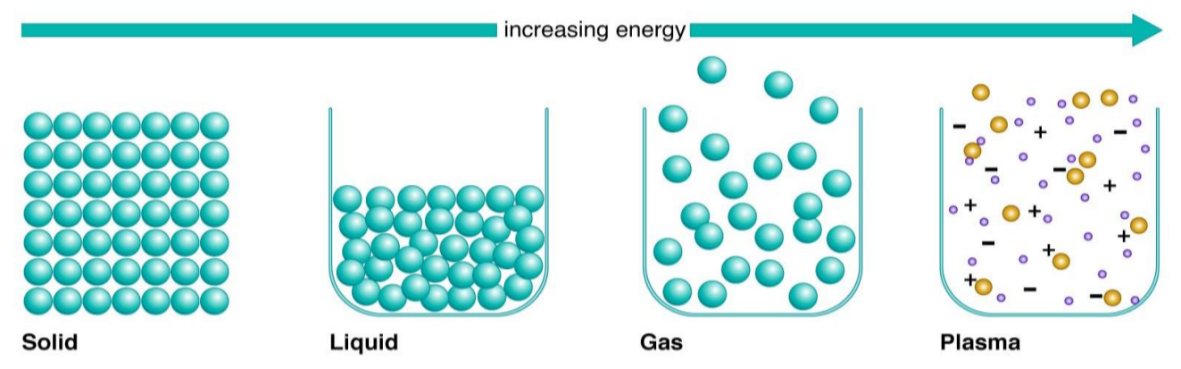


Figure 4. States of Matter. Source: (Britannica, 2024).

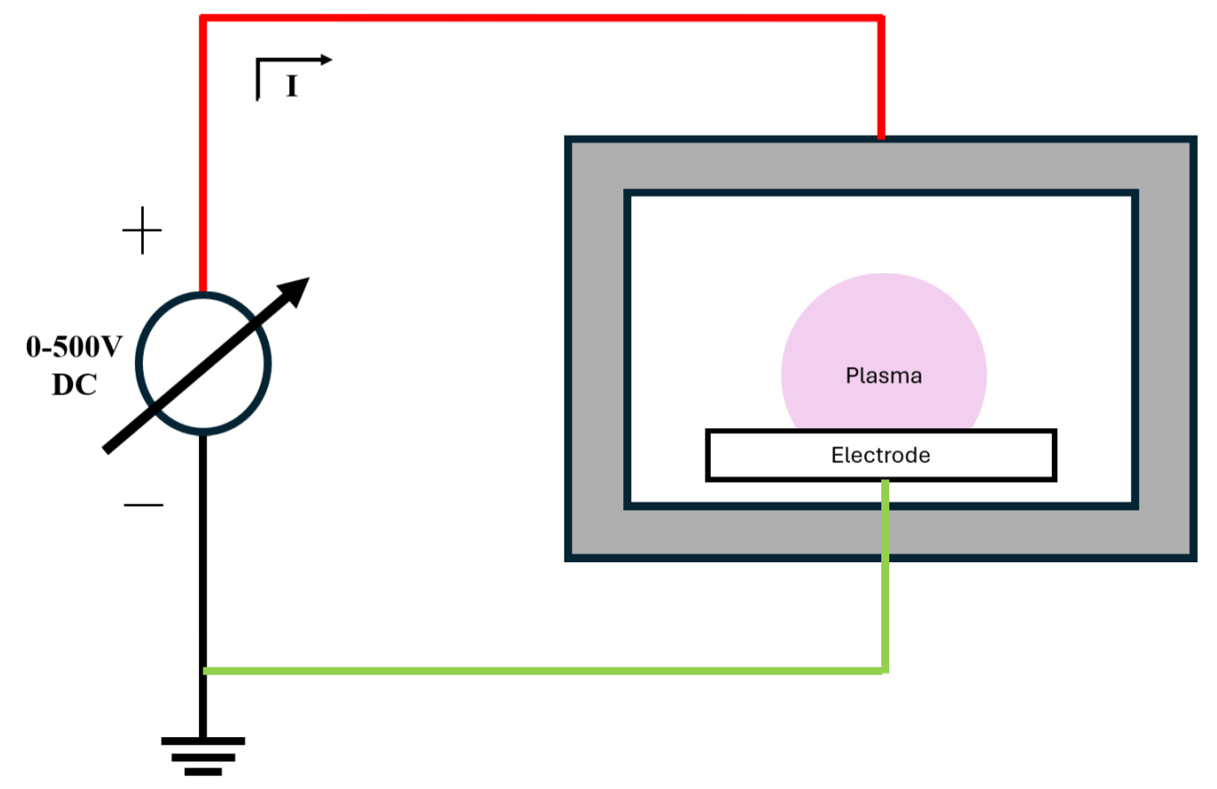
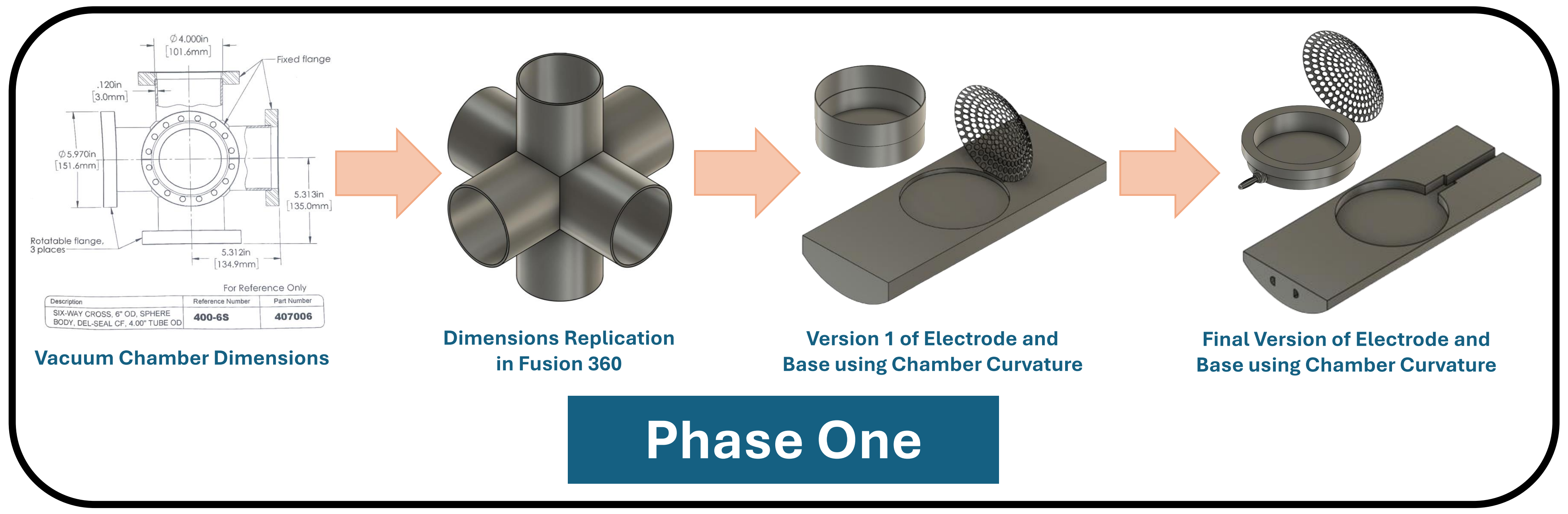


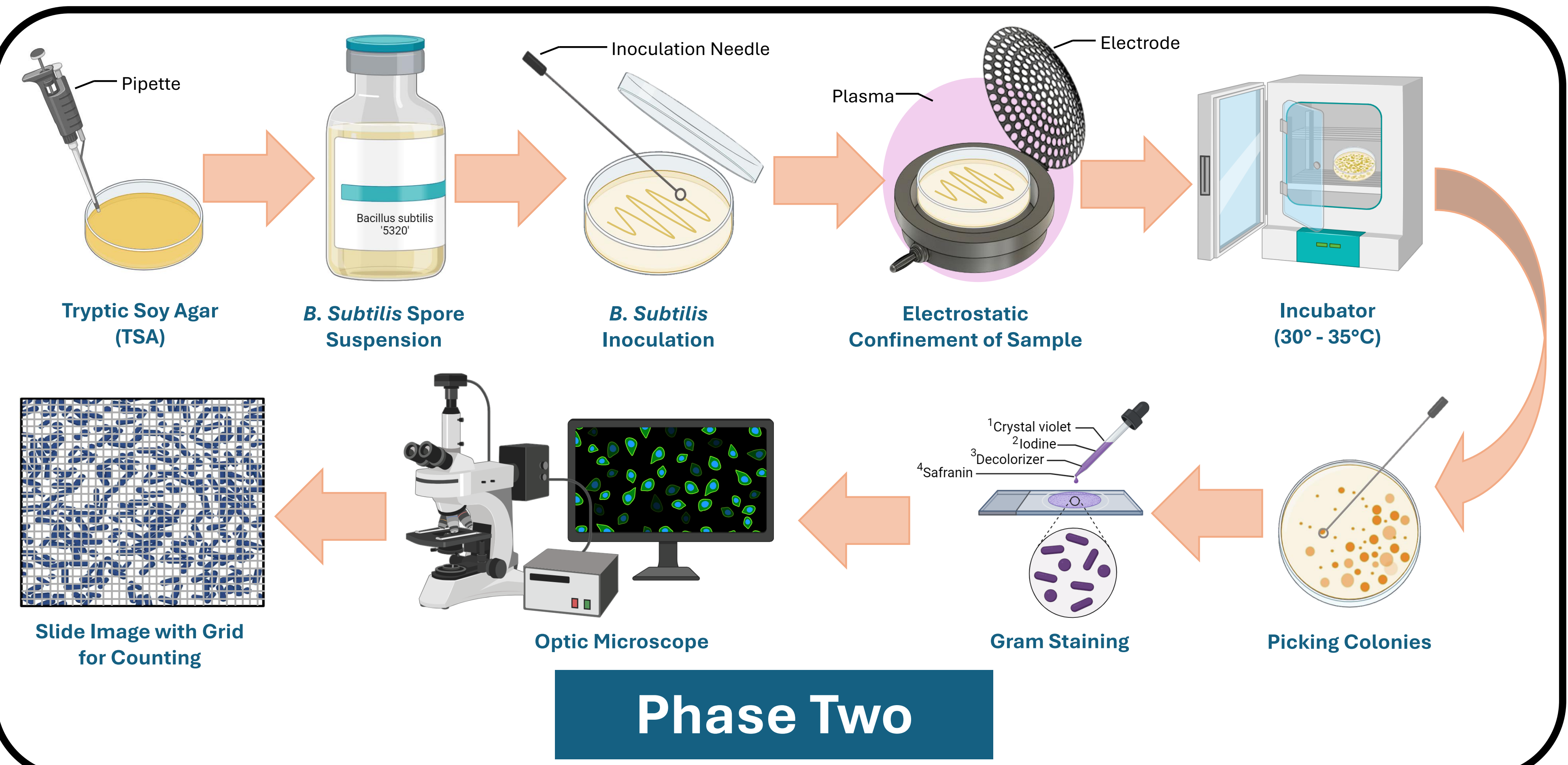
Figure 5. Electrode Interaction and Circuit Dynamics

Objectives

- Design and evaluate electrode for a concentrated plasma cloud.
- Research plasma under various voltage and pressure conditions.
- Assess the impact of vacuum conditions on TSA medium stability.

Methodology & Equipment





Results

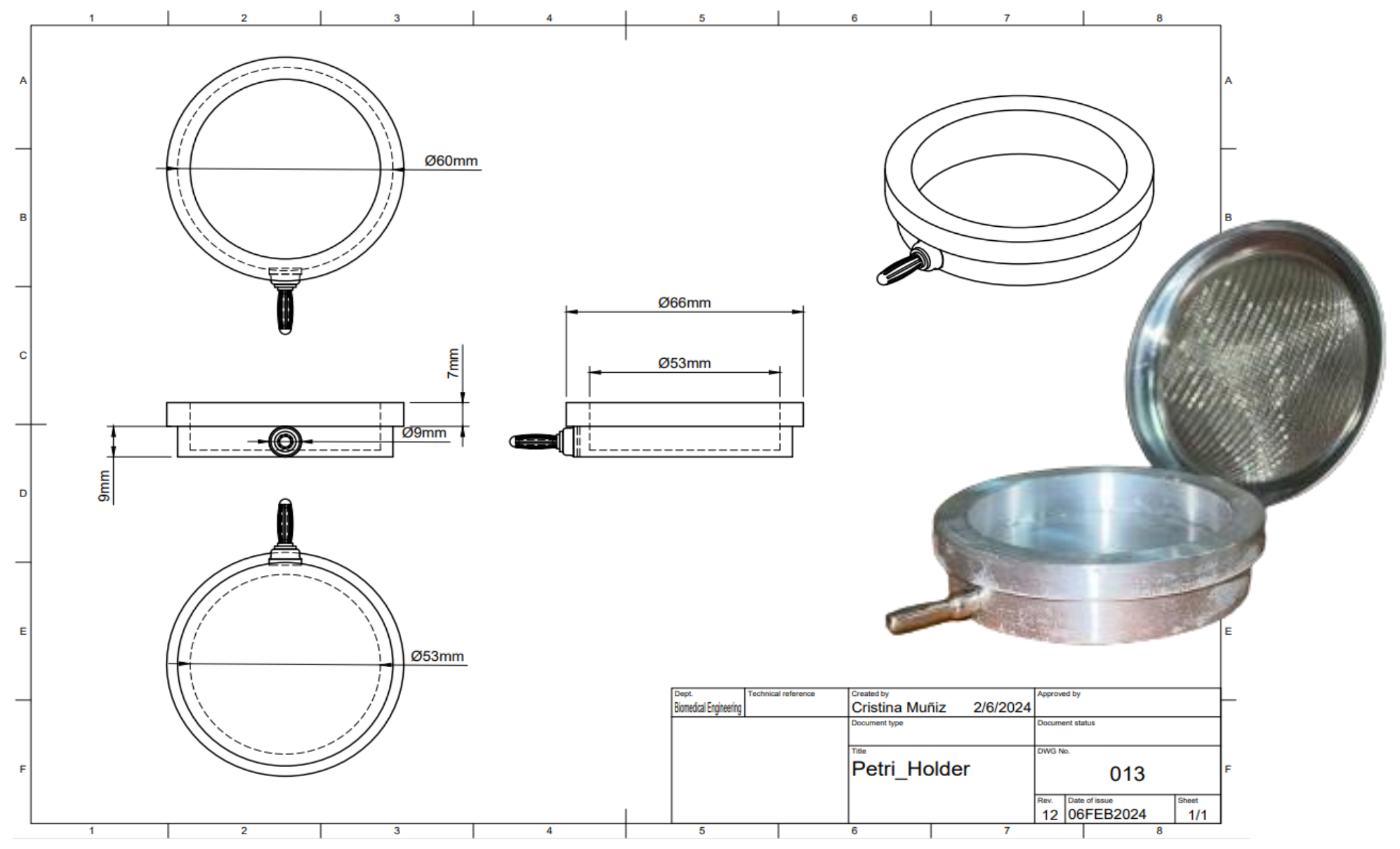


Figure 6. Electrode ('Petri_Holder') Design Drawing and Manufactured Result

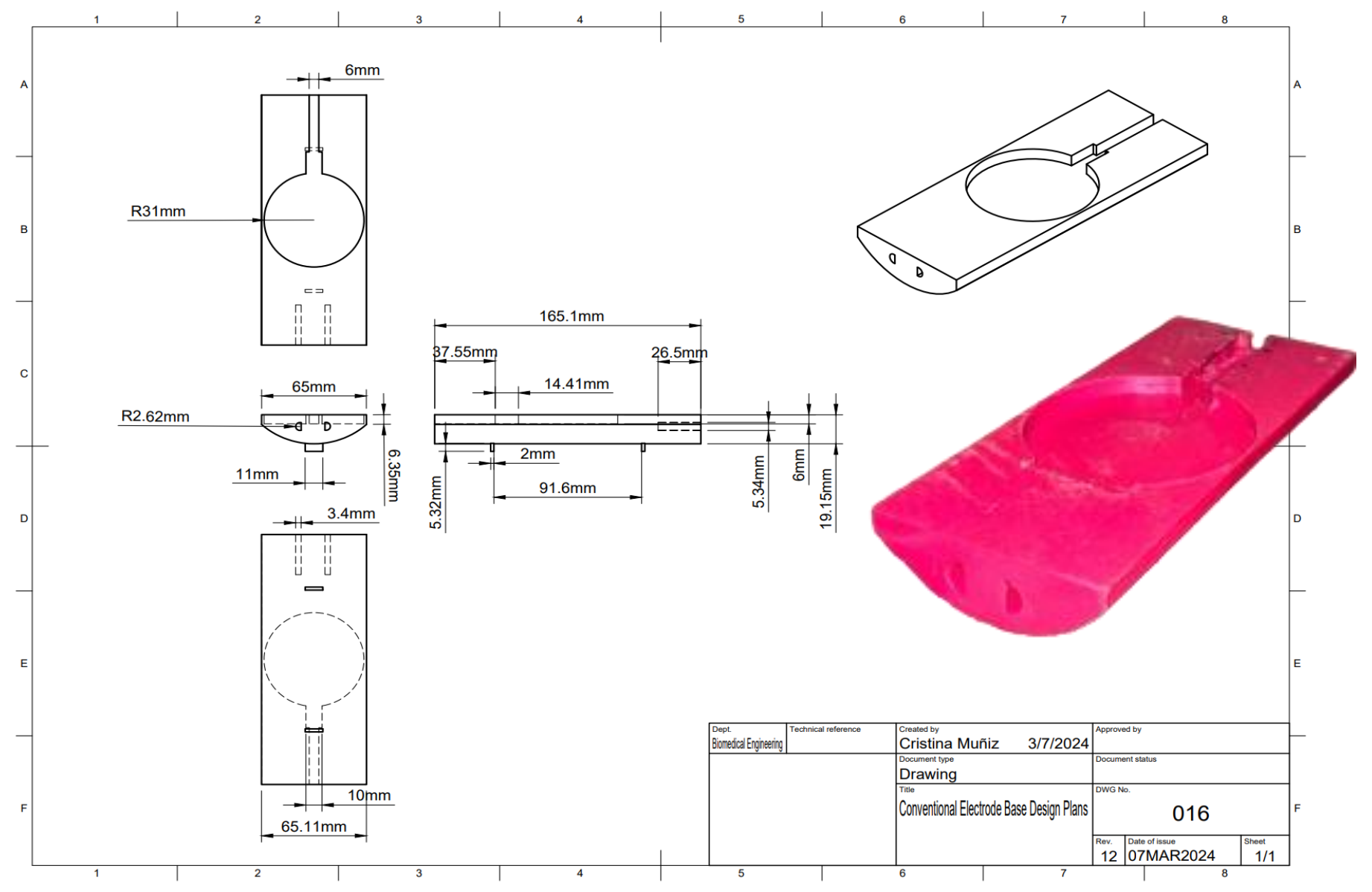


Figure 7. Electrode Base (Insulator) Design Drawing and Manufactured Result

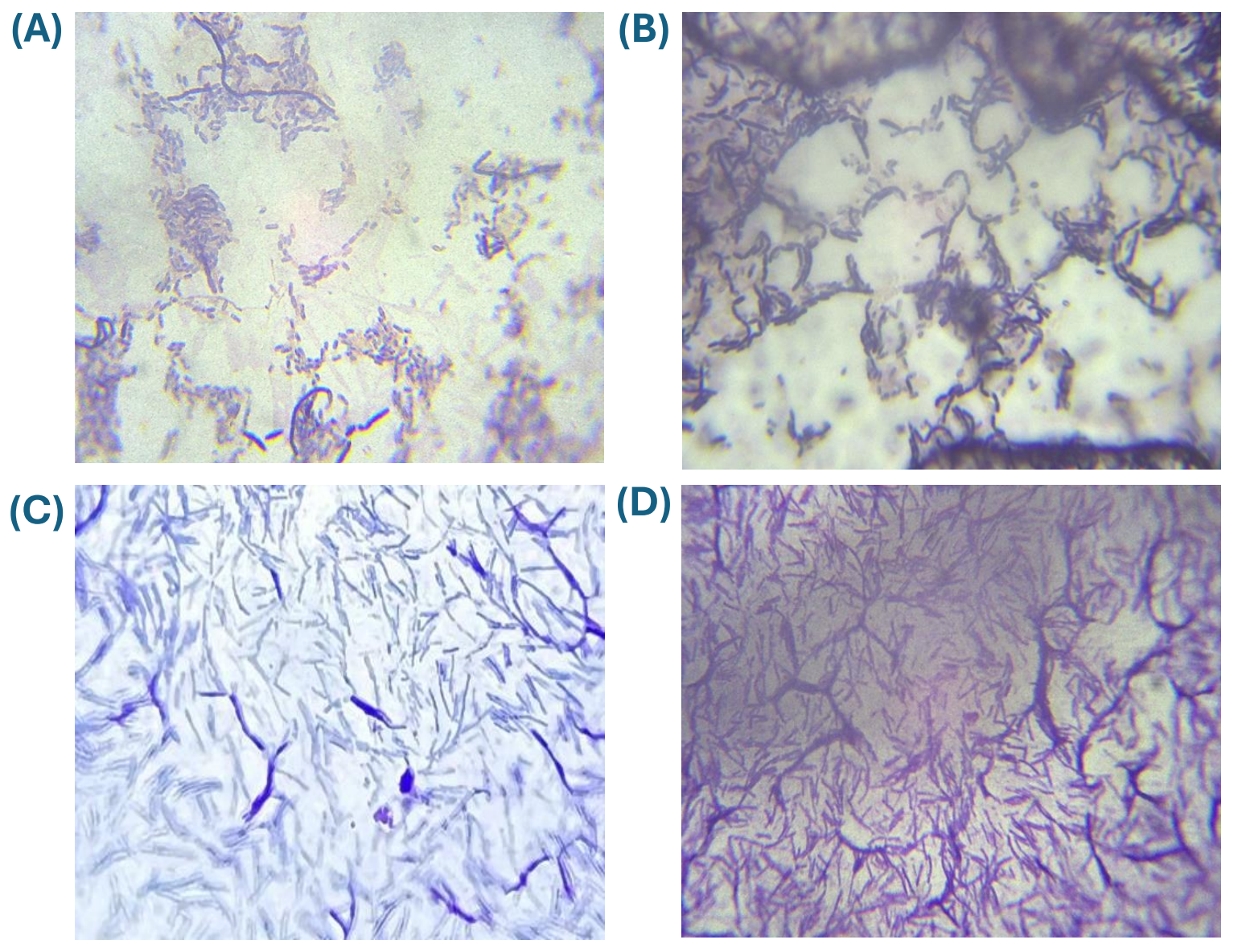


Figure 8. *B. subtilis* Gram Stain Slides (A) 2mL, (B) 3mL, (C) 4mL, (D) 6mL

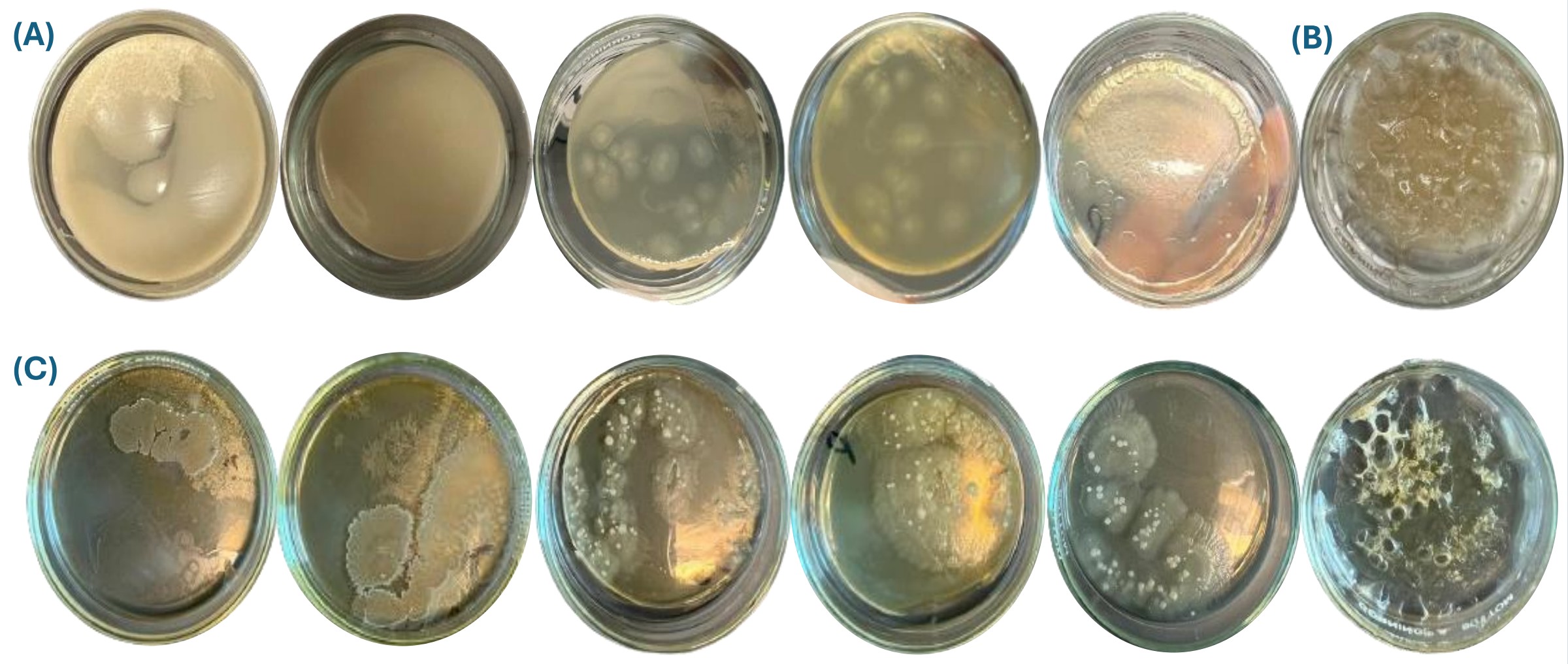


Figure 9. (A) Left to right *B. subtilis* (6mL) samples at normal solidification and plasma treatment, (B) *B. subtilis* (6mL) samples right after vacuum solidification, (C) Left to right *B. subtilis* (1mL, 2mL, 3mL, 4mL, 6mL, 6mL) samples at vacuum solidification and plasma treatment.

Analysis

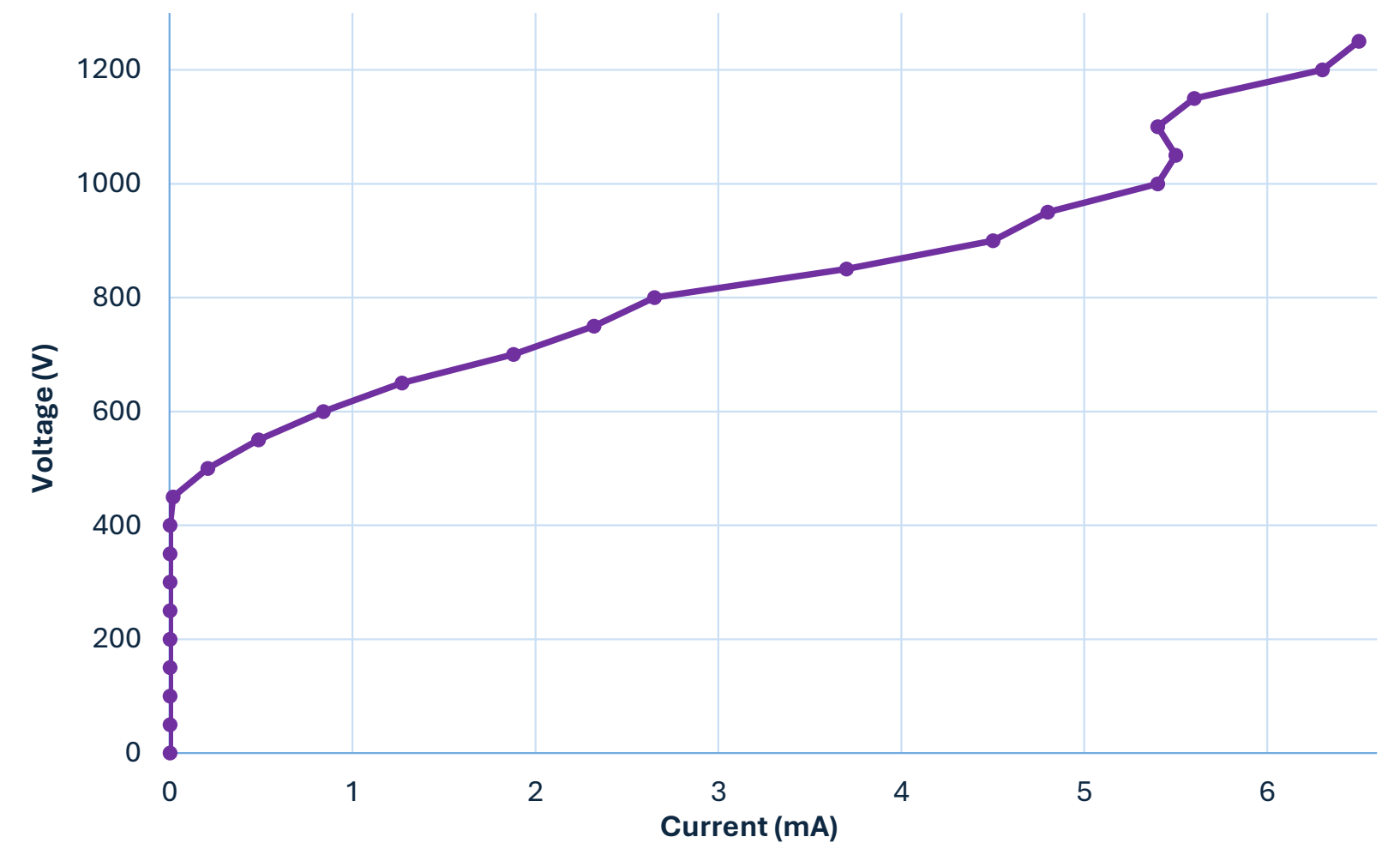


Figure 10. Relationship of Current and Voltage for Electrode Design

Volume (mL)	Solidification Method	Bacterial Growth	Plasma Treatment	Vacuum Efficiency	Observations
1	Normal	Poor (hair-like consistency)	Effective (air-based plasma)	Adequate	Volume too low for bacterial growth.
2	Normal	Poor (slightly thicker)	Effective (air-based plasma)	Slightly Low	Improved bacterial growth, still not ideal.
3	Normal	Fair (some growth)	Effective (air-based plasma)	Low	Water accumulation in pump. TSA color change.
4	Normal	Adequate (growth)	Ineffective (water-derived plasma)	Low	TSA had gel-like after plasma treatment (froze); color change.
6	Normal	Adequate (growth)	Ineffective (water-derived plasma)	Very Low	TSA had gel-like after plasma treatment (froze)); color change.
1	Vacuum	Poor (hair-like consistency)	Effective (air-based plasma)	Slightly Low	Volume too low for bacterial growth.
2	Vacuum	Poor (hair-like consistency)	Effective (air-based plasma)	Low	Volume too low for bacterial growth. TSA color change.
3	Vacuum	Adequate (some growth)	Effective (air-based plasma)	Low	Water accumulated in pump. TSA color change, slightly froze.
4	Vacuum	Adequate (growth)	Ineffective (water-derived plasma)	Very Low	TSA color change after plasma treatment. Water accumulated in vacuum pump (oil dilution).
6	Vacuum	Ineffective (no growth)	Ineffective (water-derived plasma)	Very Low	TSA had bubbles after plasma treatment. Water accumulated in pump (oil dilution).

Figure 11. Impact of TSA Volume and Solidification on Bacterial Growth, Plasma, and Vacuum Efficiency

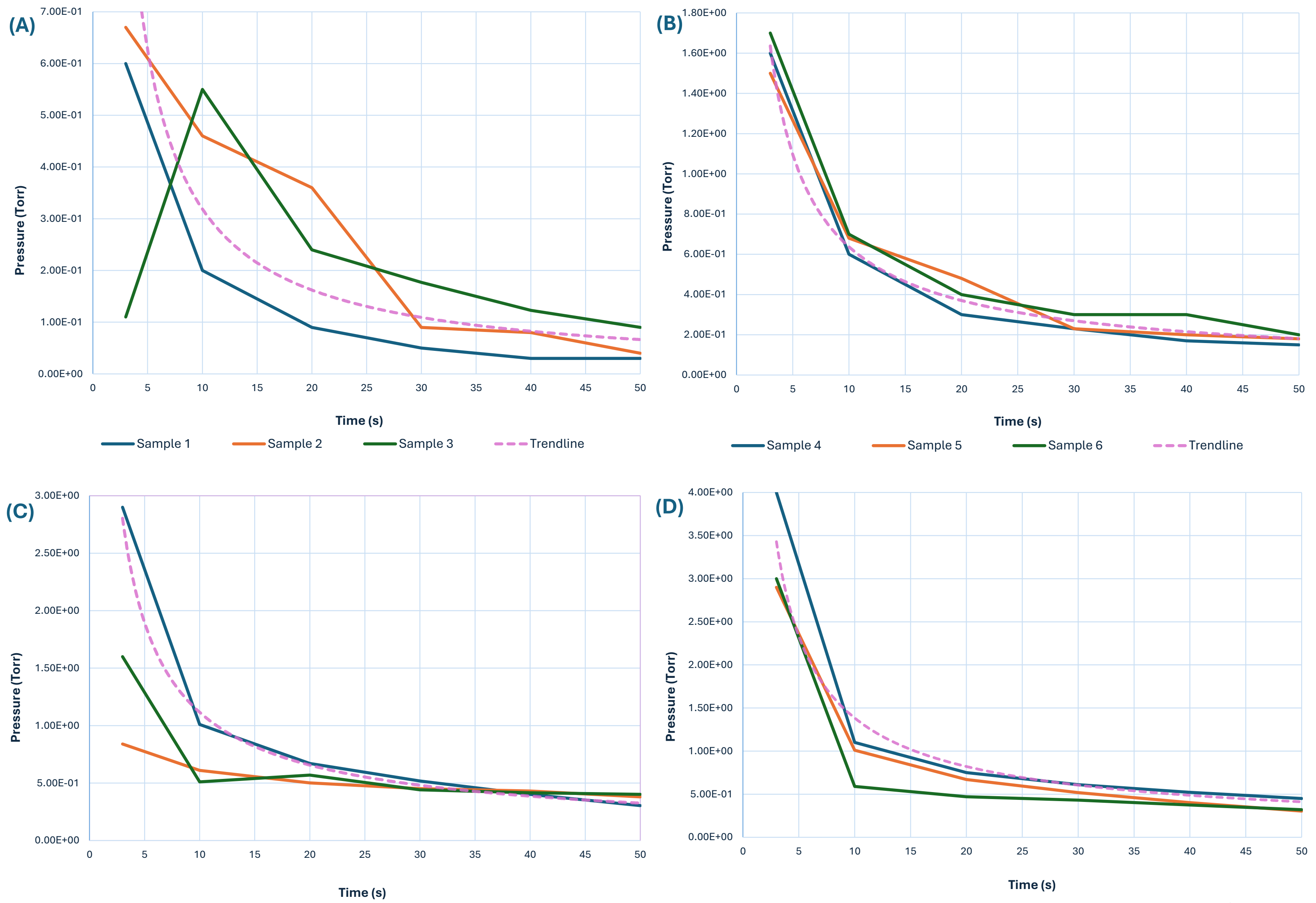


Figure 12. Time vs Pressure Relationship for Vacuum Solidified TSA Samples (A) 1mL, (B) 2mL, (C) 3mL, (D) 6mL

Conclusion & Recommendations

The experiments investigated the impact of TSA solution volumes (1mL to 6mL) on vacuum performance and plasma generation, with a TSA-to-water ratio of 1:25. At 6mL, TSA froze into a gel-like consistency, resulting in plasma sourced from evaporated water rather than ambient air, which diminished vacuum efficiency. Smaller volumes, such as 1mL and 2mL, resulted in a paper-like TSA consistency that may hinder *B. subtilis* growth due to inadequate nutrient availability rather than plasma exposure. Samples with 3mL volume produced air-based plasma but still faced low vacuum times, and 4mL samples exhibited similar issues as the 6mL samples. Attempts to solidify TSA in the vacuum chamber before evacuation did not resolve these issues. Therefore, these findings suggest that both TSA volume and its interaction with the vacuum system are critical. Further optimization of TSA formulation or vacuum system adjustments are necessary for effective plasma generation and reliable experimental outcomes. Additionally, further research should explore polymers subjected to plasma to better understand their interactions and effects, like the polymer (PLA) used for the base in this study.

Acknowledgements

We would like to express our gratitude to our mentors, Dr. González and Dr. Rondón, for their guidance and support throughout this research. We also wish to thank manufacturing instructor Joseph Mulero and Maldonado Welding for their assistance in bringing our electrode design to fruition. Our appreciation extends to professor Garriga and the Mathematics and Science Department for providing their time and equipment. Special thanks to the laboratory technicians Rey Mendez and Ashley Delestre for their support in the plasma and 3D-printing laboratories. Finally, we are grateful to the Honor committee for this experience and their guidance in navigating the research environment.

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