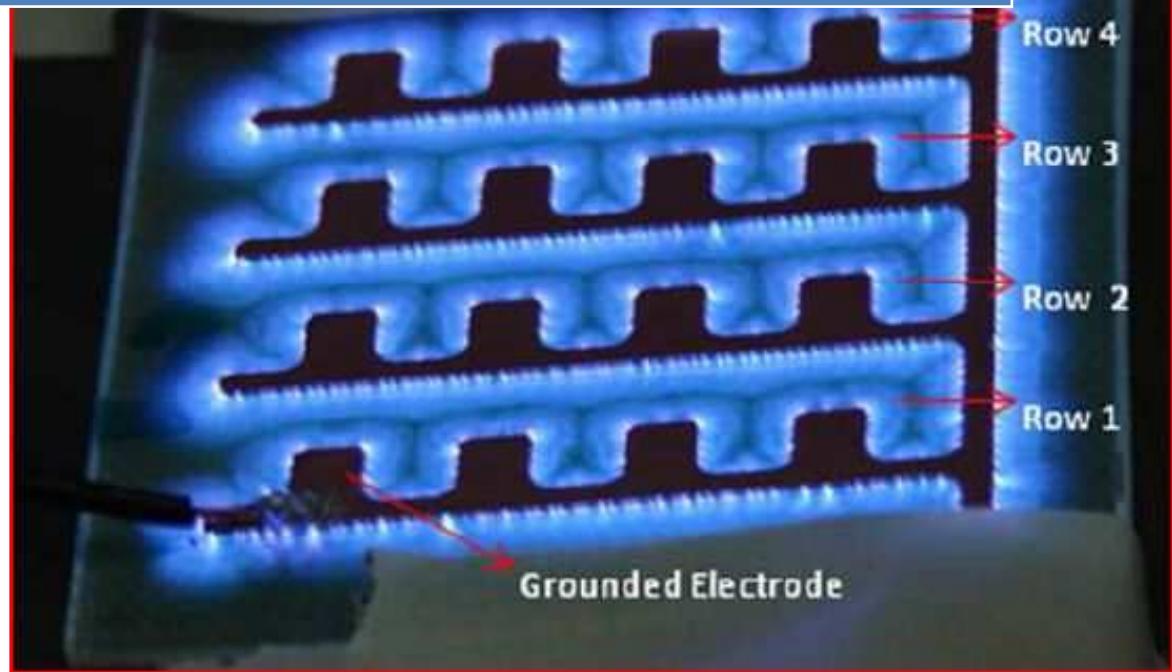


2010

Undergraduate Research: Plasma Sterilization



Abstract

This report details the test results and brief summary of my experiences participating in undergraduate research as a member of the Applied Physics Research Group, led by Dr. Subrata Roy, studying the sterilizing effects of Dielectric Barrier Discharge Plasma on yeast. This undergraduate research project provided a valuable vehicle for experiencing advanced research methodology and data collection techniques in a controlled laboratory environment. The laboratory experiments, results, and conclusions related to the topic of sterilizing effects of plasma on yeast are presented herein.

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Introduction

During the 2010 Spring Semester, I had the opportunity to participate in undergraduate research under the supervision of Dr. Subrata Roy. The focus of this research experience involved studying the effects of Dielectric Barrier Discharge Plasma on yeast, specifically, the killing effects, resulting in sterilization of the surface. This research involved participating as a member of the Applied Physics Research Group, a multi-disciplinary team consisting of mechanical engineers, electrical engineers, and biologists.

From a “big picture” perspective, the plasma sterilization project is very inspiring given the potential for application utility. That is, to be able to simultaneously activate a plasma source, and two minutes later an entire surface or countertop is completely sterile, poses enormous potential benefits due to the fact that sterilization is necessary in a wide variety of disciplines and applications.

I was initially introduced to the topic when Dr. Roy spoke about his research to our finite element analysis class in the fall of 2009, and was immediately very interested. My personal engineering interests include heat transfer and fluid analysis, which led to my initial curiosity about this fourth state of matter called plasma. I had previously heard of the concept of plasma, but hadn’t studied it to any length. After speaking with Dr. Roy, I have to say that I did initially have second thoughts about becoming involved in this specific project, simply because I have little to no background in biology. The University of Florida Mechanical Engineering curriculum does not require even an introductory biology course, and my high school biology experience can be described as sub-par, at best. In hindsight, I am glad my initial reservations did not deter me from participating on this project. The biology team was very knowledgeable and patiently explained the important biological aspects of the experiment.

Navya Mastanaiah, a PhD student working on this project, has been an amazing teacher/mentor to me throughout the semester. She was very patient in defining the background behind the endeavor and mentoring me on previous research and progress performed prior to my joining the team. Additionally, Navya allowed me to directly participate by promoting hands-on exposure in the lab and actually running experiments, as opposed to just letting me observe, which I’ve later discovered, is common of my peers experience during their undergraduate research experiences. Navya inspired a lot of critical thinking and research, and I learned so much from working with her. The Applied Physics Research Group is doing great things and it was an honor to be a part of the experience, one for which I am very grateful.

Background Research

The definition of sterilization is any process that destroys all of the micro-organisms clinging to a surface. Conventional methods of sterilization include autoclaving, Ethylene Oxide treatment, and gamma irradiation, each of which possesses numerous disadvantages. Autoclaving, which is the primary method of sterilization used in hospitals, requires long sterilizing times (up to 40

minutes) and extremely high temperatures and pressures. Ethylene Oxide treatment can take up to four hours due to the slow dissipation rate of the EtO vapors. It is a rigorous process and the threat of toxic residue makes this approach very unfavorable. Gamma irradiation is completely unpractical on all accounts. Plasma sterilization is faster, safer, and more versatile than all three of these conventional methods. Literature on previous studies proves that plasma sterilization is far superior to conventional methods in terms of exposure time and safety [1].

Plasma sterilization dates all the way back to 1968. Research has continually developed since then and scientists and engineers are beginning to understand more and more about the process.

To understand the plasma sterilization process, the concept of actual plasma needs to be explained first. Plasma is defined as the fourth state of matter and is composed only of ions and electrons. It is created by applying an electric field to a neutral gas, which ionizes the gas into charged radicals, neutrals, and UV photons. There are a number of different types of plasmas that can be obtained. The type of plasma that results is a function of the magnitude of the voltage that is applied and the discharge current [1].

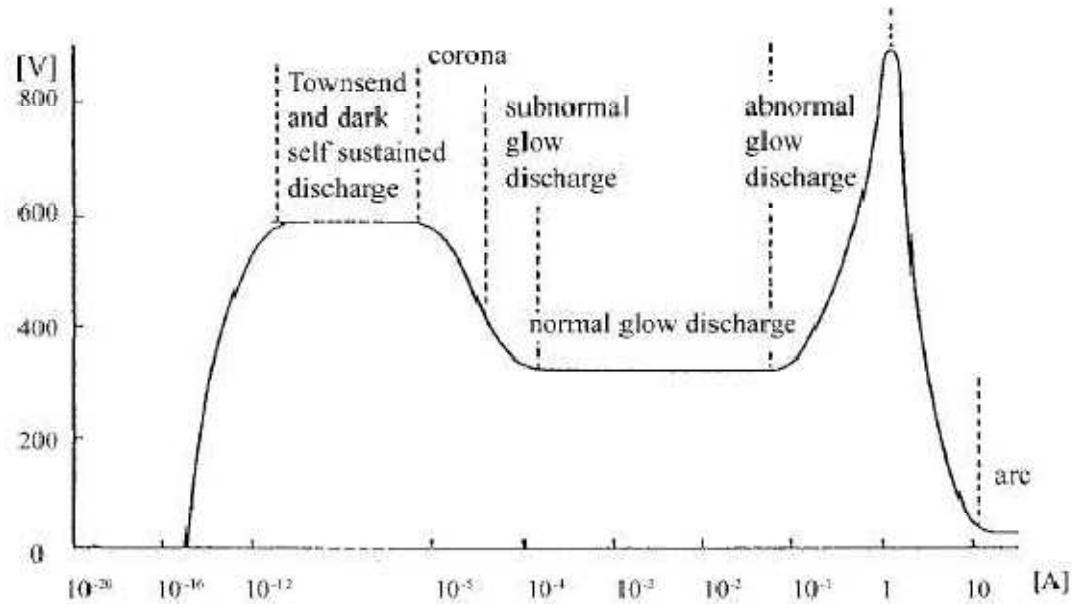


Figure 1: Dependence of voltage upon current for various types of plasma. Photo Credit: [1].

Figure 1 displays the voltage and current range for each type of plasma. The Dielectric Barrier Discharge (DBD) plasma, which is the type of plasma used in this study, occurs in the transition between the corona and the normal glow discharge. DBD plasma is generated by applying high voltage across two electrodes with a dielectric barrier in between them[1].

Although the concept of plasma sterilization has been around for awhile, the actual killing mechanism is not completely understood, although there are many hypotheses. Further research will be done to investigate the role each of the three killing agents (UV photons, charged

radicals, and neutrals) have in the process. Survival curves, which show the number of survivors as a function of time, for plasma sterilization processes are tri-phasic, as opposed to linear curves obtained in conventional sterilization operations. The UV photons initially irradiate the top layer of micro-organisms, causing a sharp drop in concentration, which correlates to the first phase of killing. The secondary lag phase and the final phase consist of the subsequent layers of micro-organisms being eroded by the UV photons and other reactive species. Figure 2 displays the survival curve obtained by the experiments that were run before I joined the team and compares it to the survival curve obtained by Jin Ying et al. during a similar experiment. The Tri-phasic killing can be observed[1].

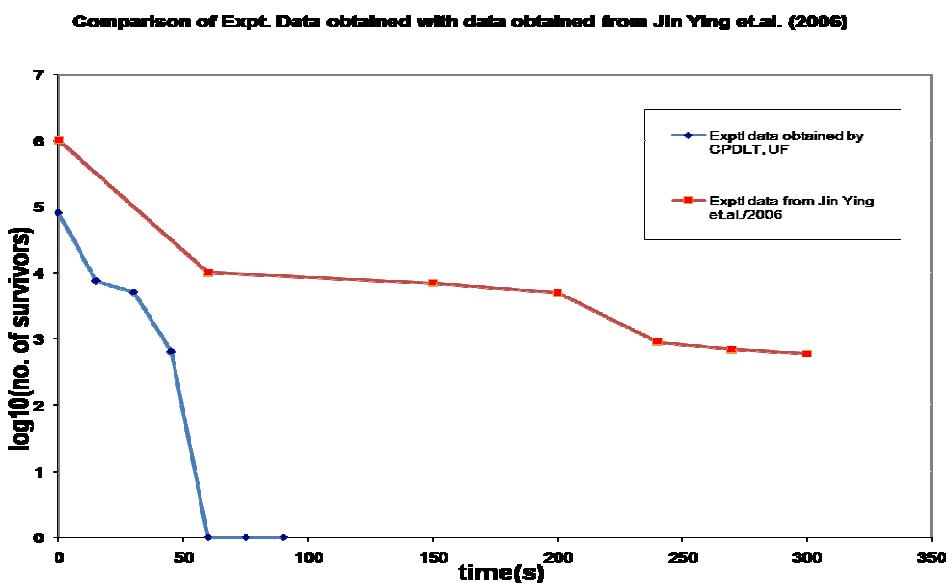


Figure 2: Survival curves obtained by Navya Mastanaiah et al. and Jin Ying et al. for Dielectric Barrier Discharge plasma on yeast. Photo Credit: Navya Mastanaiah.

After much background research, I definitely got a good grasp on the concepts and theory behind the plasma sterilization effort and continued to be fascinated by it. I was anxious to get into the lab and see some of these experiments take place and obtain a better understanding of how these results were being obtained.

Experiments

Two types of experiments were completed throughout the semester: dilution tests and stamp tests. Dilution tests are quantitative experiments performed to obtain a survival curve. The dilution tests consisted of applying a known concentration of yeast onto the device, allowing it to dry, and exposing it to plasma for various time intervals. To determine how many yeast cells survived the killing effects of the plasma, the device was thoroughly washed in broth and a dilution series was prepared so that the quantity of the resulting yeast cell survivors was a

countable number. The dilutions were then spread evenly onto agar plates, which were incubated at 37°C for 48 hours. The following figure displays the results of a quantitative dilution test.

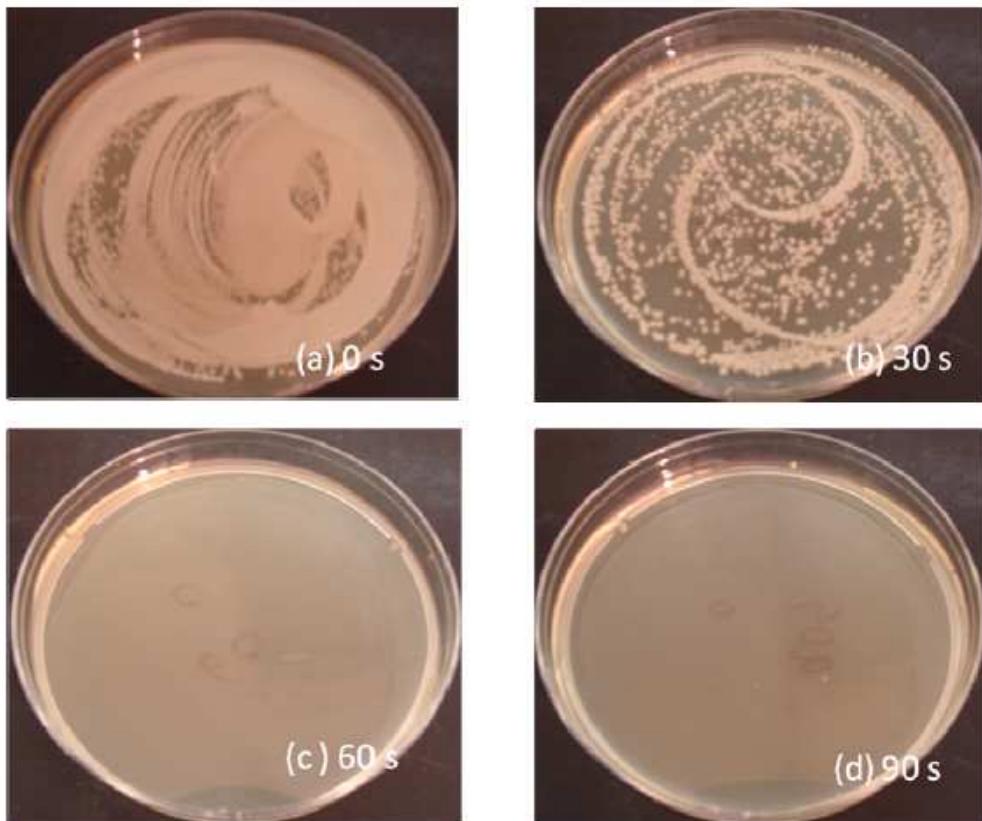


Figure 3: Yeast samples after exposure to 0, 30, 60, and 90 seconds respectively. Photo Credit: [1].

It is clear to see from the photograph that complete sterilization appears to have occurred after 60 seconds of plasma exposure for this particular experiment.

The second type of experiment performed this semester was the stamp test. The stamp test is a qualitative analysis, for which the objective is to observe exactly where the yeast cells were being killed on the plate and where the plasma was least effective. These experiments were performed by applying a yeast sample to the device, allowing it to dry, and exposing it to plasma for various time intervals. Instead of washing it in broth, however, the devices were then “stamped” directly into the agar plate facedown. Sufficient pressure was applied to allow for as much yeast as possible to transfer off of the device onto the plate. The plates were then incubated at 37°C for 48 hours, as in the previous experiment. The following figure displays the results of the qualitative stamp test.

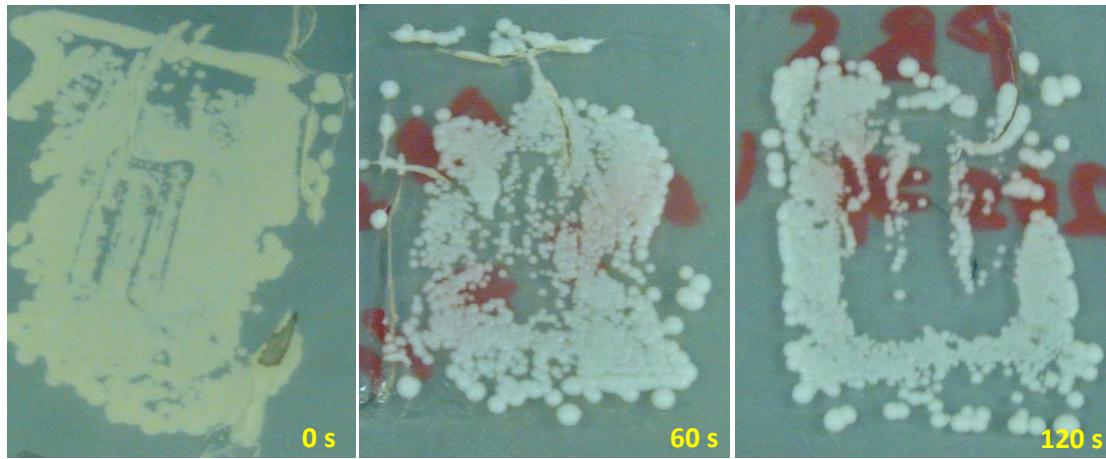


Figure 4: Yeast samples after exposure to 0, 60, and 120 seconds, respectively. Photo Credit: Navya Mastanaiah.

The electrode pattern used in this test was two straight lines directly down the center of the device. It is clear to see from these pictures that the plasma was not affecting the yeast directly on top of the electrodes, even after 120 seconds of exposure. This was an important conclusion, and plans for future experiments consist of minimizing the area of the electrode in an effort to maximize the amount of killing.

Conclusion

The concept of Plasma sterilization has been known since the late 1960's. Research has continually developed since then and scientists and engineers are beginning to gain an appreciable understanding of the process. The potential utilities for industry applications are significant. As research continues to discover, the exposure time, thickness of the dielectric barrier, and area of the electrodes are critical factors in the results of the effect.

Editorial Footnote

The undergraduate research experience has been very positive and rewarding. The result of this experience has really broadened my perspective and has helped make me a more complete engineer. I can honestly say that I definitely learned more from participating in this project than I would have if I taken a random “technical elective” class to fulfill a degree requirement. I would absolutely recommend to other undergraduates that they get involved in research. I also believe that the university and advisors need to encourage students to participate in research projects. I think a common misconception is that undergraduates perceive research as limited to graduate students only. Working on a research project really added a lot to my undergraduate experience and I am very thankful to Dr. Carroll for suggesting that I participate.

I would like to conclude this report with a very special thanks to the entire Applied Physics Research Group, and specifically Dr. Subrata Roy and Navya Mastanaiah for the effort they put in to making this a very positive experience for me this semester. It is appreciated more than you know.

Works Cited

[1] Mastanaiah, N., Saxena, U., Johnson, J., and Roy, S., "Inactivation of Yeast cells using Dielectric Barrier Discharge," *AIAA Journal*, 4-7 January 2010, AIAA-2010-1340