#### STERILISATION BY ECR PLASMA

Selcuk HELHEL Dublin City University, Plasma Research Laboratory, Dublin IRELAND Lutfi OKSUZ Dublin City University, Plasma Research Laboratory, Dublin IRELAND Abbas Yousifi RAD S. Demirel University, Faculty of Science – Biology TURKEY Osman CEREZCİ Sakarya University, Electrical and Electronics Eng. TURKEY

### ABSTRACT

Microwave plasma was built to produce plasma in axial direction. Plasma was initiated in a Plaxy Glass made vacuum tube by 2.45GHz commercial magnetron and meanwhile system was driven by 14 Amperes DC current passing through 16cm inner diameter toroid.

Measurements with a Longmuir probe and ICCD for optical spectrometry were used to characterize internal parameters like electron density, electron temperature and different properties of the heavy particles. This study presents the bacteria disinfection capability of ECR based plasma system. Those are gram positive and gram negative bacteria that refers to structure of cell wall. The sterilization efficiency of Microwave plasma was found to be over 99, 5 % in Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis (vegetative cell), Bacillus cereus (vegetative cell), Klebsiella pneumonia and Escherichia coli.

### I. INTRODUCTION

Sterilization process of heterogeneous materials, particularly those containing polymer pieces, need not only be efficient in destroying micro-organisms but must also work at low temperatures [1]. Achieving such a process at relatively low temperatures ( $\leq 50$  °C) preserves the integrity of polymer-based instruments. Since autoclaving and subjecting them to ovens is impossible, EtO is the alternative method to be treated rather than cold plasma techniques.

D-value which represents the number of survivals drops  $10^6$  to 1 is a measure of sterilization capability of equipment that is being used by scientists [1-2-3]. In order to talk about real sterilization, we have to achieve total inactivation of bacillus. That's why different kinds of bacillus some have spores have been chosen. Staphylococcus aureus, Staphylococcus epidermidis, Bacillus subtilis (vegetative cell), Bacillus cereus (vegetative cell), Klebsiella pneumonia, MRPA and Escherichia coli type bacteria were treated at the beginning. Then the most resistant bacteria type was chosen and study was focused on them. Bacillus cereus and bacillus subtilis are very resistant to microwave heating.

Because of their higher resistance, they are generally accepted as reference bacteria for proving any sterilization capability of equipment.  $N_2 O_2$  and Argon – Oxygen mixture were treated by V. Monna [1] and M. Maison [4] used before, and pure Argon and He plasmas were treated.

#### II. EXPERIMENTAL SETUP

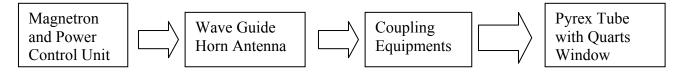


Figure1. Block Diagram of Sterilization Equipment.

The block diagram of the setup is represented in Figure1 and the experimental setup is reproduced in Figure3. Argon and Helium discharges were generated in Plaxy Glass tube. Microwave inlet part of tube was constructed by quartz window and fixed by an additional stainless steel adaptor. Tube diameter is 100mm outer diameter and 330mm in height. The gas flow rate typically between 0.3-2.5 Liters per minute and the gas pressure in the reactor is between 0.07 torr to 0.4 torr. Substrate holder is 2.5cm by 20 cm Pyrex. The Petri dish (lam) is located at 15cm from one side. The magnetic field strength generated by coils at this location is 87.5mTesla that is matching with 2.54GHz magnetron frequency. This magnetic field strength decreases up to 80mTesla because of cooling problems of coils during test period. Two pieces can be treated simultaneously on the substrate holder. The reactor chamber is connected /fixed to the discharge tube. There is an inlet for substrate holding has a diameter of 3cm. Substrate holder temperature during process is measured between 25°C to 40°C. This temperature range for sterilizing heat sensitive equipments is very good and acceptable.

Figure 2 represents the drawback of designed system. It is supplied from a 2450-MHz surfacewave discharge operated at the 450–700 W power level in a 100mm diameter Pyrex tube. Pressure is in the 0.07–0.4 torr range with a gas flow rate typically between 0.3 and 2.5 standard L/min. The Petri dish is located at 15 cm from the end.

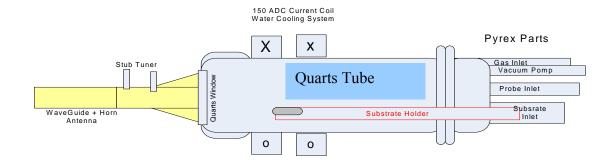


Figure2. Drawback of System

# **III. MATERIAL and METHOD**

McFarland's opacity standard was chosen at value of McFarland 0.5. This level is enough for colony forming units per milliliter (CFU/ml). All objected bacteria samples were cultured during 16 hours at  $37^{0}$ C. After incubation was completed, final concentration of bacteria suspension reached to McFarland 0.5 in saline 0.85%, and Crystal Spec Nephelometer of Becton Dickenson was used as suspension value measurement. Following bacteria were prepared to test ECR plasma equipment.

- A. Bacillus Subtilis var Nigar
- B. Bacillus Cereus
- C. E Coli
- D. MRSA

In sterile conditions, silicone catheter were cut into 20 pieces that each by 2cm long. They were infected by using those prepared suspension at 100rpm during 60minutes. Finally, they were removed from suspension, and washed gently three times. Saline 0.85% was used for washing process. After all these processes were completed, those pieces were treated by plasma discharges.

# IV. INACTIVATION OF BACTERIA

For applied microwave power levels sterilization time is different for all bacteria types as shown below in Figure 3. Alphabetically assigned bacteria at Figure 3 and Figure 4 were given before. Table1 represents the reactivation duration of Bacillus Cereus if any. Each combination was repeated by 20 times. Average reaction time and real sterilization time responses are given in Figure3 and Figure4.

Close results were detected for Bacillus subtilis and Bacillus Cereus as Q.Wu [5]. These two bacteria are the more resistant than the others for heating and microwave. Rest two bacteria types have no spores and they are easy to kill. Comparing the results given in Figure 3 and Figure 3, we can see that Helium sterilization time is slightly less than Argon plasma. This is because of Helium ionization capability is easier and faster than Argon.

		Power	
		(Minutes)	
Treatment Time (Minutes)		350	700
	5	YES	YES
	10	YES	YES
	15	YES	YES
	20	YES	YES
	25	YES	YES
	30	YES	YES
	35	YES	NO
	40	NO	NO
L	50	NO	NO

Table 1. Re activation of Argon Plasma Treated Silicone Catheter after 24 hours.

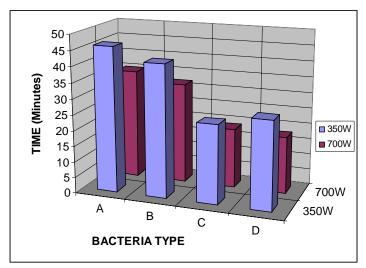


Figure 3. Argon Plasma Sterilization Response

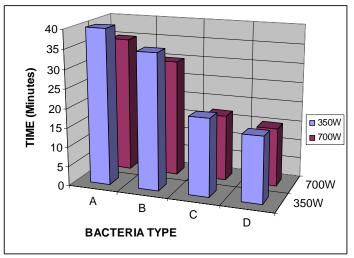


Figure 3. Helium Plasma Sterilization Response

# V. CONCLUSION

The investigations shown and the results presented prove that ECR plasma is able to inactivate bacteria. Inactivation time is less than an hour and that's why it is a real alternative sterilization technique.

The sterilization time depends on bacteria type and applied microwave power. During this study, microwave coupling has been assumed as perfect that no microwave reflected back to magnetron. But it is not true. There are some mismatches causing power losses. Matched microwave systems are well coupled to plasma cabinet to ionize more efficiently. Efficient ionization will increase the plasma density that will most probably disinfect bacteria. Since improving coupling efficiency will most probably decrease sterilization time, a coupling efficiency of ECR plasma systems and its resulted effects on bacteria disinfection will be held on as our next study.

Other gases such as Nitrogen, Oxygen and Hydrogen and their different mixtures will also be treated for next applications to achieve less sterilization time by lower cost.

## VI. ACKNOWLEDGEMENT

This study was supported by DCU – Plasma Research Laboratory (Ireland), and experiments were held on S.Demirel University (Turkey).

### REFERENCES

[1]. V. Monna, C. Nguyen, M. Kahil, A. Ricard, and M. Sixou, Sterilization of Dental Bacteria in a Flowing N2–O2 Post discharge Reactor, IEEE TRANSACTIONS ON PLASMA SCIENCE, VOL. 30, NO: 4, AUGUST 2002 1437.

[2]. Mounir Laroussi, Igor Alexeff, Biological Decontamination by Thermal Plasmas, IEEE TRANSACTIONS ON PLASMA SCIENCE, VOL. 28, NO.1, February 2000, page 184-188.

[3]. I. A. Soloshenko, V. V. Tsiolko, V. A. Khomich, V. Yu. Bazhenov, A. V. Ryabtsev, A. I. Schedrin, and I. L. Mikhno Features of Sterilization Using Low-Pressure DC-Discharge Hydrogen-Peroxide Plasma, IEEE TRANSACTIONS ON PLASMA SCIENCE, VOL. 30, NO. 4, AUGUST 2002.

[4]. Michel Moisan1,<sup>‡</sup>, Jean Barbeau2, Marie-Charlotte Crevier3, Jacques Pelletier4, Nicolas Philip1, and Bachir Saoudi1 Plasma sterilization. Methods and mechanisms, Pure App. Chem., Vol. 74, No. 3, pp. 349–358, 2002.
[5]. Q.Wu, Effect of High – Power Microwave on Indicator Bacteria for Sterilization, IEEE Transactions on Biomedical Engineering, Volume.43, No.7, July 1996.