# Plasma sterilization – special features and new approaches in medical applications

**Kiril Ivanov**

*An overview of the process of sterilization and disinfection is described in this article. Special attention is turn to the currently used technologies, their advantages and disadvantages. Another subject of the topic is the plasma and its basic principles. It is crucial to understand the process of forming plasma, and all particles that are created. Only after understanding the process of creating plasma, we will be able to understand the plasma-based disinfection and sterilization. The main emphasis of this article is the status of plasma sterilizations technology and the future of it. At the end, the best approaches for moving the plasma sterilization to the next level are discussed.*

***Keywords – Disinfection, Plasma, Sterilization.***

***Стерилизация с плазма – особености, медицински приложения и нови подходи (Кирил Иванов).*** *В настоящата статия е направен обзор на процесите на стерилизация и дезинфекция. Специално внимание е обърнато на съществуващите технологии, техните предимства и недостатъци. Разгледано е физичното явление плазма и неговите основни принципи. Описани са процеса на генериране на плазмата и различните частици, които се получават при него. Разяснени са процесите на дезинфекция и стерилизация чрез плазма. Основна цел на настоящата статия е запознаване и оценка на съществуващите плазмените стерилизатори. В края на темата са обсъдени и възможните подходи за бъдещо развитие на тези устройства.*

Disinfection and sterilization are both decontamination processes. While disinfection is the process of eliminating or reducing harmful microorganisms from inanimate objects and surfaces, sterilization is the process of killing all microorganisms. Sterilization also destroys the spores of various organisms present on surfaces, in liquids, in medication, or in compounds such as biological culture media. Such "extreme" forms of decontamination are needed during surgery, or in environments like industrial, laboratory or hospital. It is more practical to use disinfection in everyday life.

**Methods of sterilization and disinfection.**

Disinfection is usually carried out by usage of disinfectants (chemicals). Some of them may be very effective and have a wide spectrum while others may have a narrow spectrum but, they may be easy to use, be non toxic or inexpensive.

Sterilization can be done by three main methods: physical, chemical and physiochemical. Physical method includes heat, radiation, and filtration. Chemical methods involve usage of liquid and gaseous chemicals. Physiochemical is a combination of physical and chemical method.

There are few types of disinfection processes:

- Air disinfectants – disinfectant is dispersed as either an aerosol or vapor at a sufficient concentration in the air.
- Alcohols – high-concentration can effectively inactivate viruses such as HIV, hepatitis B, and hepatitis C.
- Aldehydes – are somewhat effective on spores and fungus also.
- Oxidizing agents – cause the microorganisms to collapse. Chlorine and oxygen are strong oxidizers, so their compounds are used for e.g. common household bleach.
- High-intensity shortwave ultraviolet light are used to disinfect smooth, opaque materials.

There are five main methods for sterilization:

- Steam – used in machines called autoclaves. Autoclaves use steam heated to 121 - 134℃ (250 - 273°F) for predefined amount of time.
- Heating – under heating flaming, incineration,

boiling in water, tindalization, dry heat. These methods inactivate and kill microorganisms in objects like glass, metal.

- Chemical sterilization - Chemicals like Ethylene oxide, Ozone, Bleach, Glutaraldehyde and Formaldehyde, Phthalaldehyde, Hydrogen Peroxide, Peracetic acid and Silver are used in varying degrees. Products that can get damaged due to heat are subjected to chemical sterilization for e.g. biological materials, fiber optics, electronics, and plastics.
- Radiation sterilization - Electron beams, X-rays, gamma rays, or subatomic particles are used for sterilizing disposable medical equipment.
- Sterile filtration - Clear liquids that would be damaged by heat, irradiation or chemical sterilization can be sterilized by mechanical filtration [1].

**Functional parameters of sterilizers**

Many different sterilizers are present on the market today. Most common of these sterilizers are using moist or heat sterilization. Moist heat may be used in three forms to achieve microbial inactivation:

- Dry saturated steam – Autoclaving;
- Boiling water / steam at atmospheric pressure;
- Hot water below boiling point.

Moist heat sterilization involves the use of steam in the range of 121-134°C. Steam under pressure is used to generate high temperature needed for sterilization. Saturated steam acts as an effective sterilizing agent.

Most common device is the autoclave. Model of an autoclave is shown on the figure below (Figure 1).

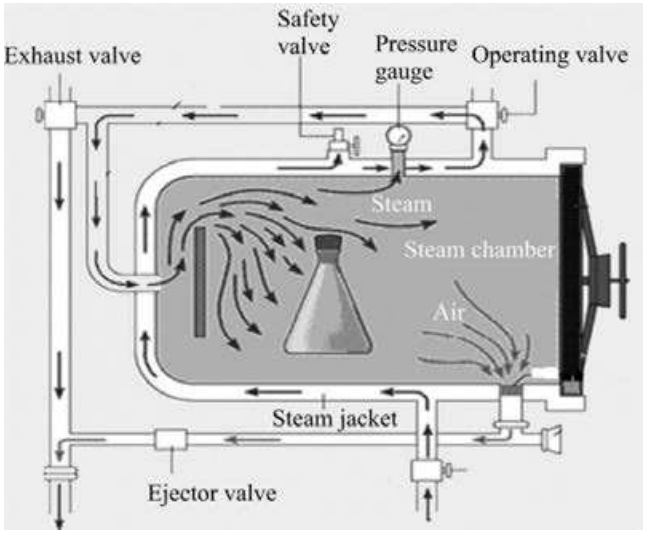


*Figure 1. An Autoclave.*

Autoclaves use pressurized steam to destroy microorganisms, and are one of the most dependable systems available for the decontamination of laboratory waste and the sterilization of laboratory glassware, media, and reagents. For efficient heat transfer, steam must flush the air out of the autoclave chamber. This method of sterilization works well for many metal and glass items but is not acceptable for rubber, plastics, and equipment that can be damaged by high temperatures.

All of the autoclaves have the same operation process. For porous loads (dressings) sterilizers are generally operated at a minimum temperature of 134°C for one hour, and for bottled fluid, sterilizers employing a minimum temperature of 121°C are used. The stages of operation of autoclaves include air removal, steam admission and sterilization cycle (includes heating up, holding/exposure, and cooling stages) [2].

Another type of sterilizer is the microwave sterilizer. Microwave sterilizer machine use temperature from 70°C to 105°C, period is from 90 to 180 seconds. Microwave sterilization equipment thermal effects change the bacterial protein and make it lose nutrition, reproduction and survival conditions and death. Microwave electromagnetic field can make normal growth and stability of the genetic breeding of bacteria nucleic acid [RNA] and deoxyribonucleic acid [DNA] number of hydrogen bonds slack, breakage and recombination, thereby inducing genetic mutations, chromosomal aberrations and even rupture. Microwave sterilization machine can fast sterilize under low temperature sterilization. It is used for sterilize rice, spices, snack and different food material.

Next method is by ultraviolet germicidal irradiation (UVGI). UVGI is a disinfection method that uses short-wavelength ultraviolet (UV-C) light to kill or inactivate microorganisms by destroying nucleic acids and disrupting their DNA, leaving them unable to perform vital cellular functions. UVGI is used in a variety of applications, such as food, air, and water purification. UV offers a reliable, cost effective, environmental friendly alternative to chemicals and their resulting bi-products. The effectiveness of germicidal UV depends on the length of time a microorganism is exposed to UV, the intensity and wavelength of the UV radiation, the presence of particles that can protect the microorganisms from UV, and a microorganism's ability to withstand UV during its exposure.

There are manufactures of UV sterilizers for medical facilities, pharmaceutical production, municipal reclaimed water, aquaculture and drinking water. One of the main reasons for usage of UV sterilization, especially for water, is that the

sterilization is fully chemical free.

One of the newest and most interesting methods for sterilization and disinfection is by using plasma. For understanding of the plasma sterilization method, first it is needed to be understood the plasma itself.

**Basics of the plasma**

Plasma is often called the "Fourth State of Matter". Although found in virtually every home and business, gas plasma is not well known. In fact, plasma is quite common - it is estimated that 99% of the visible universe consists of plasma. The term plasma was first introduced by chemist Irving Langmuir in the 1920s. He proposed the following description: “A plasma is a quasi-neutral gas consisting of positively and negatively charged particles (usually ions and electrons) which are subject to electric, magnetic and other forces, and which exhibit collective behavior”.

Plasma can be simply considered a gas of charged particles. Taken as it is, this definition is not especially useful and, in many cases proves to be wrong. Yet, necessary properties come from it: (a) presence of freely moving charged particles, and (b) large number of these particles. These charged particles are negatively charged electrons and highly charged positive ions, being created by heating a gas or by subjecting gas to a strong electromagnetic field. However, true plasma production is from the distinct separation of these ions and electrons that produces an electric field, which in turn, produces electric currents and magnetic fields [4].

Stripping away electrons from atomic nuclei achieve the positive charge in ions. The number of electrons removed is related to either the increase in temperature or the local density of other ionized matter. This also can be accompanied by the dissociation of molecular bonds, though this fundamental process is distinctly different from chemical processes of ion interactions in liquids or the behavior of ions existing in metals. A significant number of highly charged particles together make plasma electrically conductive so that it responds strongly to electromagnetic fields [5].

Three factors are listed in the definition of a plasma stream:

- The plasma approximation: Charged particles must be close enough together that each of them influences many nearby charged particles, rather than just interacting with the closest ones (these collective effects are a distinguishing feature of plasma).
- Bulk interactions: The Debye screening length (number of charge carriers within the sphere of influence, called the Debye sphere whose radius is the Debye screening length) is short compared to the physical size of the plasma.
- Plasma frequency: The electron plasma frequency (measuring plasma oscillations of the electrons) is large compared to the electron-neutral collision frequency (measuring frequency of collisions between electrons and neutral particles).

Depending of the temperature, there are two types of plasma – high and low temperature plasma. High temperature plasma is more often on the Earth. Typical representative in the nature are lightning. The sudden electrical discharge causes ionization of the atmospheric gas, creating plasma (lightning). High temperature plasma is easy to create artificially by using very high voltages. This is also the base of the corona effect and the plasma torches, used for etching and deposition.

Low temperature plasma is used for surface etching, disinfection, sterilization etc. It is mostly created by ionization of gases in vacuum or low pressure environment, inside chambers with controlled environment in them – atmospheric gases are absent or have very low concentration. The absence of other gases, allow the particles accelerated by the plasma creation to have very long collision free trajectory inside the chamber. Since the temperature of these particles is equal to the environmental temperature, and the rate of collisions is very low, this allows the achievement of low temperature plasma.

Recently low temperature, high pressure, non-equilibrium plasmas are used in several material processing applications, and in some cases are competing with low pressure plasmas in areas where these have historically been dominant. Amongst the novel applications of non-equilibrium plasmas, biomedical applications such as electrosurgery, surface modification of biocompatible materials, and the sterilization of heat-sensitive medical tools are particularly interesting. A brief overview of recent research plasma-based sterilization/decontamination methods is given [3,6].

Biological materials can be exposed to plasma in two different methods: “Direct exposure” is when the sample to be treated is in direct contact with the plasma. All plasma-generated agents, including charged particles, come in contact with the sample. The second method is “remote exposure”. In this case the sample is placed at a distance from the plasma volume or in an adjacent chamber. In this configuration, the amount of heat transmitted to the sample is reduced, the charged particles do not play a

role since they recombine before reaching the sample, and many of the short-lived neutral reactive species also do not reach the sample. In the following section contribution of the four main inactivation factors of non-equilibrium high-pressure air plasma are reviewed.

*Heat:* heat-based sterilization methods use either moist heat or dry heat. In the case of moist heat, such as autoclave (its operation was described above) a high temperature and high pressure is used. Dry heat sterilization requires temperatures close to 170°C and treatment times of about 1h.

To assess the inactivation role of heat from high pressure non-equilibrium air plasma, some researches have been done. The gas temperature in the discharge was determined, by comparing experimentally measured rotational bands structure of the 0–0 transition of the 2nd positive system of nitrogen with simulated spectra at different temperatures. By using a thermocouple probe, it was also measured the temperature in a sample, placed 2 cm away from the discharge.

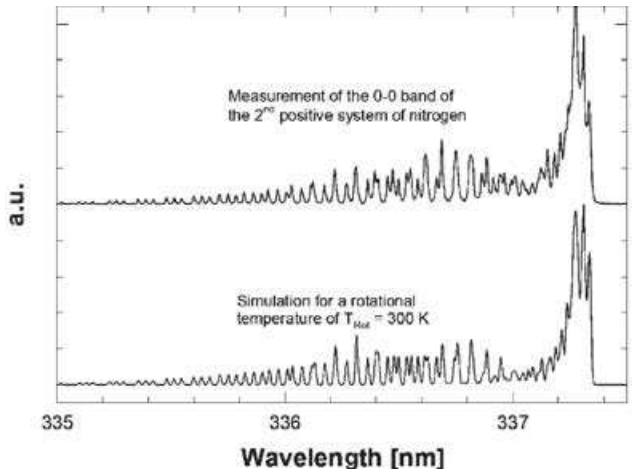


*Figure 2. Measured and calculated rotational bands of 0-0 transition of the second positive system of nitrogen. The spectra are intentionally shifted vertically for better comparison [7].*

Figure 2 shows the measured and calculated rotational bands of the 0–0 transition of the 2nd positive system of $N_2$, for a power of 10 W. It indicates that the gas temperature remains close to room temperature. A variation in power from 2W to 15W showed no variation in the temperature. An air flow rate of 10 l/ min was used in these experiments. The gas temperature for various gas flow rates at a power level of 10W was also investigated. The results are shown in Figure 3.

Increasing the airflow causes the gas temperature to approach room temperature (300 K). *Figure 4* shows the increase in the temperature of the biological sample under treatment for various dissipated power levels, as measured by a thermocouple. A maximum increase of 21K was observed. Therefore, based on these measurements no substantial thermal effects on bacterial cells are expected [3], [6].

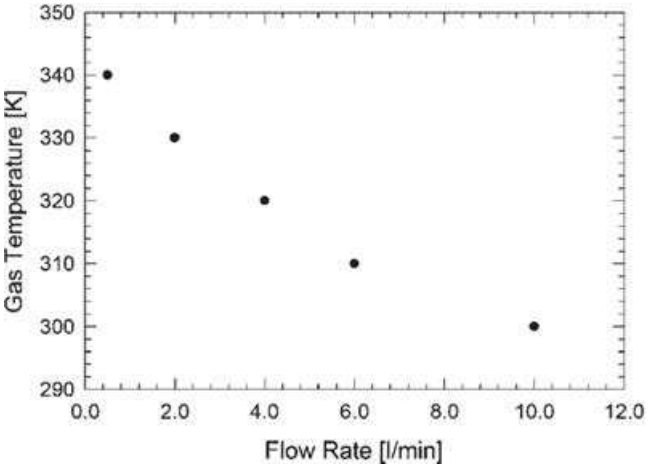


*Figure 3. Gas temperature versus gas flow rate for power of 10W [7].*

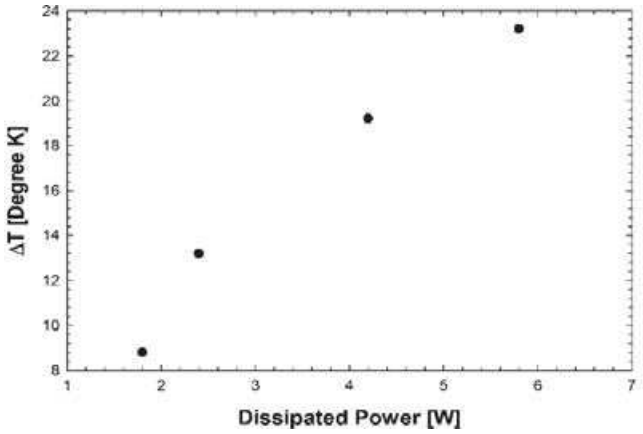


*Figure 4. Increase of sample temperature versus plasma dissipated power.*

*UV Radiation:* UV radiation in the 200-300nm wavelength range with doses of several mW·s/cm$^2$ causes lethal damage to cells. Amongst UV effects on cells of bacteria is the dimerization of thymine bases in their DNA strands. This inhibits the ability of the bacteria to replicate properly [9].

To evaluate the UV contribution to the inactivation process of non-equilibrium air plasma, spectroscopic and absolute power measurement was conducted. This research showed that no significant UV emissions occur below 285nm. This is illustrated on Figure 5. Power measurement with calibrated UV detector in the 200-300nm wavelength region revealed that the power density of the emitted UV radiation does not

play a significant direct role in the sterilization process by low temperature air plasmas [3], [6].

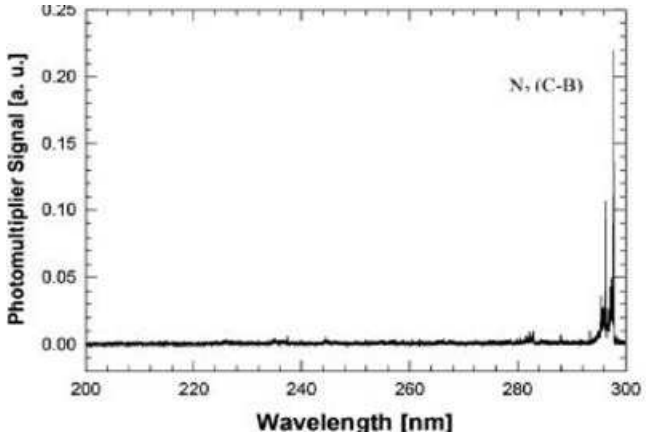


*Figure 5. UV spectrum of a DBD in air in the 200-300nm wavelength range [7].*

*Charged particles:* Charged particles play a very significant role in the rupture of the outer membrane of bacterial cells. Electrostatic force caused by charge accumulation of the outer surface of the cell membrane could overcome the tensile strength of the membrane and cause its rupture.

When charged, a body of the size of a bacterial cell (in the μm range) experiences an outward electrostatic force because each charge is subjected to the repulsive forces of all the similar changes accumulated on the cell surface. This force is proportional to the square of the charging potential, $\Phi$, and inversely proportional to the square of the radius of the curvature of the surface, $r$. Therefore, the smaller the radius of curvature the stronger the electrostatic force. The charging potential $\Phi$ depends on the ratio of the ion mass to the electron mass. So gases with larger atomic mass lead to higher electrostatic forces [10]. Based on this the condition for membrane disruption is:

$$|\Phi| > 0.2 \cdot (r \cdot \Delta)^{1/2} \cdot F_t^{1/2}, \quad (1)$$

where $r$ is the radius of the curvature, $\Delta$ is the thickness of the membrane, and $F_t$ its tensile strength [8].

The scenario described above is more likely to occur for gram-negative bacteria, the membrane of which possesses an irregular surface. These irregularities offer small radii of curvatures that cause localized high outward electrostatic forces [3,6].

*Reactive Species:* In high-pressure non-equilibrium plasma discharges, reactive species are generated through various collisional pathways, such as electron impact excitation and dissociation. Reactive species play an important role in all plasma-surface interactions. Air plasmas, for example, are excellent sources of reactive oxygen-based and nitrogen-based species, such as O, $O_2$, $O_3$, OH•, NO, $NO_2$, etc.

Oxygen-based and nitrogen-based reactive species have strong oxidative effects on the outer structures of cells. Cell membranes are made of lipid bilayers, an important component of which is unsaturated fatty acids. The unsaturated fatty acids give the membrane a gel-like nature. This allows the transport of the biochemical by-products across the membrane. Since unsaturated fatty acids are susceptible to attacks by hydroxyl radical (OH•), the presence of this radical can therefore compromise the function of the membrane lipids whose role is to act as a barrier against the transport of ions and polar compounds in and out of the cells. Protein molecules are susceptible to oxidation by atomic oxygen or metastable oxygen molecules. Proteins also play the role of gateways that control the passage of various macromolecules in and out of cells. In the case when bacteria are of the gram-positive type, they are able to form spores, which are highly resistive states of cells. Spores are made of several coats surrounding a genetic core. These coats are also made of proteins susceptible to chemical attack. Therefore, the reactive species generated by air plasmas are expected to greatly compromise the integrity of the walls, coats, and membranes of the cells of microorganisms.

Low-pressure plasmas have been considered for biological sterilization for some time. Some of the systems developed in the 1970s and 1980s were not really "plasma-based" sterilization systems. This is due to the use of gas mixtures that contain components with germicidal properties (such as $H_2O_2$ and aldehydes) before the plasma is ignited. These are more correctly termed as "plasma-assisted" sterilization systems. Plasma-based sterilization uses gases that possess no germicidal property on their own. They become biocidal only when a plasma is ignited. Example of such gases or mixtures of gases are air, helium/air or helium/$O_2$, and $N_2$/$O_2$ [3], [6].

Most recently, many studies on the effects of low-pressure plasma on biological matter in plasma-based systems were conducted for various gas mixtures. Examples are low-pressure oxygen plasmas and $O_2$/ $N_2$ plasmas. RF and microwave driven low-pressure plasmas were mostly used in these studies. Recently a detailed study of the effects of RF oxygen plasma at reduced pressure on bacteria was published. The study was carried out for two modes of operation, the inductively coupled mode and the capacitively coupled mode. The inductive mode was found to offer a better efficiency in destroying biological matter.

This was due to higher electron and ion densities in this mode, which resulted in an enhancement of electron-impact processes. High densities of atomic oxygen and perhaps $O_2$• in synergy with UV photons induced chemical degradation of the biological materials followed by volatilization of the decomposition products ($CO_2$, CO, $N_2$ etc.). Plasmid DNA degradation was evaluated for both the inductive mode and the capacitive mode. It was found that at the same power the inductively coupled plasma destroyed over 70% of supercoiled DNA in 5 s while only 50% was destroyed by the capacitively coupled plasma. Characterization of the decomposition of the by-products was carried out during plasma exposure by emission spectroscopy. CO, $N_2$, $N_2^+$, OH, Na, K etc. were amongst the detected species [11].

In the early studies on the inactivation of Bacillus subtilis spores by low-pressure plasmas, it was reported that survivor curves exhibited three inactivation phases. First phase, which exhibited the shortest D-value (Decimal value is the time required to reduce an original concentration of microorganisms by 90%), was mainly due to the action of UV radiation on isolated spores or on the first layer of stacked spores. The second phase, which had the slowest kinetics, was attributed to a slow erosion process by active species (such as atomic oxygen, O). Finally, the third phase was initiated after spores and debris had been cleared during phase 2, hence allowing UV to hit the genetic material of the still living spores. The D-value of this phase was observed to be close to the D-value of the first phase. However, in a more recent study, the same research group examined the inactivation process of B. subtilis spores exposed to the flowing afterglows of an $N_2/O_2$ mixture and of pure argon, and reported that UV radiation, not the radicals, played the dominant role. The survivor curves were biphasic and consistent with UV inactivation. The second phase represented spores that were shielded by others and that needed more irradiation time to accumulate a lethal UV dose. This observation was further supported by the fact that at low UV intensity a lag time existed before inactivation. This was due to the requirement that a minimum UV dose had to be achieved before irreversible damage to the DNA strands occurred. Since in pure argon, which would not contain oxygen radicals, inactivation was achieved for similar lengths of time, it was concluded that the role of oxygen in the $N_2/O_2$ plasma was mainly to provide oxygen atoms to form NO, which was the main source of the UV photons [3], [6].

**Further development of plasma sterilizers**

Considering all research results described in the topic, plasma-based sterilizer would have positive impact on decontamination process. Such sterilizer shall have features as:

- Small size – the sterilizer or its accessory need to be small and mobile. This will contribute for the easy use during operation on everyday basis;
- Fast decontamination effect – decontamination effect shall be achieved very fast (around 1min);
- Easy to use – it is very important for the sterilizer to be easy operated by the end user.

There are two main options that need to be considered before beginning with the design: (1) the plasma will be in contact with the object and (2) the plasma will not be in contact with the sterilized object. Most of the researches show that the main sterilization is done by the UV light and the radicals, but not by the heat of the plasma.

Most of the requirements of the sterilizer are related to the source of the output signal and the signal itself. This signal will be responsible for the plasma generation, so its parameters need to be very well selected. First thing need to be specified is the output signal frequency. RF signal with frequency above 500kHz, should offer high effectiveness. Output generator may be designed to work with frequencies up to 4MHz, but the germicidal effect of the plasma has to be examined. Next should select the waveform of the signal. All experiments conducted until now shows that the higher the selected duty cycle is, the higher the temperature of the plasma will be. If the duty cycle is 100% or close to it, the ionized gas (plasma) have no time to cool down, so the temperature gets high, and vice versa. Another thing that depends on the waveform of the signal is the output signal peak voltage. If the selected waveform is with low duty cycle, then the peak voltage has to be very high. This is necessary to sustain the plasma.

After defining all output signal parameters next is to select the appropriate gas that will be used. Gas will be medium for creating plasma and shall be carefully selected. Intensity of the UV light will depend on the molecules of the gas. Also the radical created by the plasma will vary according to the gas. Best option is to select gas that is not flammable and create a lot of radicals that can affect both gram-positive and gram-negative bacteria.

Designing sterilizer that meets all of the requirements described above will have big effect on the decontamination process in general. Such device will solve many of the issues that the surgeons have today.

This paper is reported in the National forum with international participation Electronica, Sofia, Bulgaria, 2017.

**REFERENCES**


[1] Disinfect vs. Sterilize, http://www.diffen.com/difference/Disinfect_vs_Sterilize

[2] Autoclaves, http://www.explainthatstuff.com/autoclaves.html

[3] M. Laroussi, Low Temperature Plasma-Based sterilization; 2005, Wiley-YCH Verlag GmbH.

[4] Dr Peter T. Gallagher, Introduction of Plasma Physics; 2003, Trinity College Dublin.

[5] S. Schwartz. Basic Plasma Physics. Imperial College London, 1993.

[6] S. Lerouge, M.R. Werheimer. Plasma Sterilization: A review of parameters, mechanisms, and limitation, 1999.

[7] M. Larroussi, F. Leipold, Int. J. Mass Spectrom. 2004, 233, 81.

[8] D. A. Mendis, M. Rosenberg, New J Phys. 2003, 5, 41.1.

[9] A. Norman, J. Cell. Comp. Physiol. 1954, 44, 1.

[10] D. A. Mendis, M. Rosenberg, F. Azam, IEEE Trans. Plasma Sci. 2000, 28, 1304.

[11] O. Minayeva, M. Laroussi, in: Proc. IEEE Int. Conf. Plasma Sci., IEEE Press, Baltimore, MD 2004, 122.



***Kiril Ivanov***

*Tel: +359887000654*

*E-mail: kiril.sl.ivanov@gmail.com*



**Received on: 30.10.2017**