Received: 30 January 2023 | Revised: 29 March 2023 | Accepted: 30 March 2023

DOI: 10.1002/ppap.202300012

RESEARCH ARTICLE

PLASMA PROCESSES AND POLYMERS

Validation of an indirect nonthermal plasma sterilization process for disposable medical devices packed in blisters and cartons

Paolo Seri[1] | **Silvia Nici**[2] | **Martina Cappelletti**[2] | **Silvia G. Scaltriti**[1] | **Arturo Popoli**[1] | **Andrea Cristofolini**[1] | **Gabriele Neretti**[1]

[1]Department of Electrical, Electronic and Information Engineering, University of Bologna, Bologna, Italy

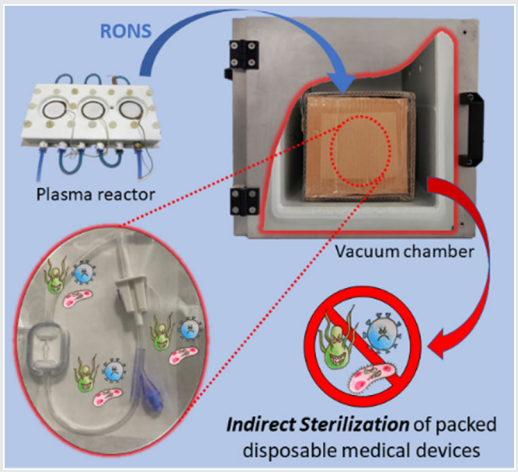
[2]Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy

Correspondence
Gabriele Neretti, Department of Electrical, Electronic and Information Engineering, University of Bologna, Bologna, Italy.
Email: gabriele.neretti@unibo.it

Funding information
Italian Ministry of Economic Development, Grant/Award Number: Proof of Concept 2019

Abstract

Nowadays, the majority of the processes used to sterilize disposable medical devices have several drawbacks in terms of safety, energy consumption, and costs. In this work, a sterilization method based on an indirect nonthermal plasma treatment is presented. The main advantages of this method are low environmental impact, absence of harmful chemical compounds' storage, and backward compatibility relative to production, sterilization, and shipping chain. The sterilization of disposable devices, enclosed inside their protective packaging, is achieved by exploiting reactive species produced by a Dielectric Barrier Discharge plasma reactor. Various devices have been subjected to a 2-h treatment, achieving complete sterilization based on USP and EUPHARMA protocols. Pretreatment of carton packaging has been necessary to guarantee a complete sterilization process.



KEYWORDS
afterglow plasma processes, disposable medical device, nonthermal plasma, ozone, sterilization

1 | INTRODUCTION

Disposable medical devices were created with the primary goal of preventing hospital-acquired infections. They are usually made of plastic materials and are utilized only once. Their use has increased significantly in the last few years, and the medical disposables market is expected to double within the next 5 years.[1] To be effectively stored and subsequently used, these devices must be sterilized inside their protective blisters. Their thermal-sensitive nature forces the adoption of cold sterilization methods. Nowadays, ethylene oxide (EtO) and radiation (Gamma rays) represent the most used methodologies for this task. More than 50% of disposable devices are sterilized with EtO.[2] This process involves the use of a treatment chamber filled with cartons containing devices to be treated (sealed in their protective blisters). The air is evacuated from the treatment chamber, to be subsequently replaced by the sterilizing gas agent (EtO). As far as the blister membrane porosity enables the passage of the gasses, the above-mentioned replacement of air by EtO can take place inside the blister allowing samples sterilization. This gas guarantees high sterilization standards even for samples characterized by complex geometries. On the other hand, the USA Occupational Safety and Health Administration claims that "EtO is both flammable and highly reactive. Acute exposures to EtO gas may result in respiratory irritation and lung injury, headache, nausea, vomiting, diarrhea, shortness of breath, and cyanosis. Chronic exposure has been associated with the occurrence of cancer, reproductive effects, mutagenic changes, neurotoxicity, and sensitization."[3] Among the existing sterilization technologies, the gamma radiation process covers almost 40% of the treated medical disposable devices.[2] Although this method also guarantees high sterilization standards, it is expensive and the radiation can be harmful to workers and can damage the material that needs to be sterilized. Other methods like the use of hydrogen peroxide are usually confined to small sterilization volumes.

The huge growth that the sterilization market is experiencing and the need for new effective, low-cost, and environmentally friendly methods are pushing both researchers and industry into exploring new sterilization technologies. Our research group recently developed a sterilizing methodology for disposable medical devices, based on a nonthermal plasma indirect treatment (Patent WO2019234781A1). The process is performed using water-cooled annular plasma synthetic jet actuators (APSJAs),[4] delivering inactivation species into a treatment chamber. In recent years, nonthermal plasmas[5,6] have been shown to be effective for pathogen inactivation, mainly due to the production of charged particles and reactive oxygen and nitrogen species (RONS).[7,8] These compounds interact with bacteria, parasites, yeasts, molds, and viruses, damaging their structures and DNA, and eventually leading to their inactivation.[8–16]

Nowadays, several different techniques exist for nonthermal plasma production at atmospheric pressure. These include microwave plasmas, ultraviolet (UV) radiation, corona discharges, gliding arcs, and dielectric barrier discharge (DBD) plasmas. Each of these techniques has several pros and cons that depend on the considered practical application-specific requirements. With respect to the other listed families, one of the main strengths of DBDs is that they are easily scalable when the needed power levels exceed those typically achieved in laboratories. Moreover, DBDs inherently prevent transitions of the discharge to arc mode, making it quite suitable from the safety standpoint.[17]

An APSJA is a particular arrangement of a Surface DBD (SDBD) actuator. This reactor configuration takes advantage of the induced wind produced by the electro hydro dynamic (EHD) interaction,[18–20] enhancing the transport of RONS and charged particles.[21,22]

The main advantages of the presented inactivation method with respect to those discussed above are as follows:

- It is backwards compatible with the EtO process, avoiding the modification of disposable packaging and manipulation, thus minimizing the impact of this new technology in the sterilization chain.
- The carrier gas is synthetic air. This gas does not present any storage and manipulation restrictions, and it is cheap and readily available.
- Sterilizing species are produced on site, avoiding transportation and storage of harmful chemical compounds, hence reducing the carbon footprint of the whole process.
- Sterilizing active species produced by the discharge can be easily filtered or abated after the treatment, minimizing impact on the environment.

This paper focuses on the description of a sterilization method applied to disposable medical devices enclosed in their protective containers. Since packaging is known to be able to affect the sterilization procedures, the considered devices are packed inside cartons which are commonly utilized to handle and deliver samples in the industrial chain.[23] The sterilization efficacy has been proven on the above-mentioned devices based on standard international protocols. These tests, performed on unknown initial microorganisms concentrations, are essential to establish the efficacy of the proposed

sterilization method and its backward compatibility with respect to existing sterilization methodologies. Moreover, known different initial microoragnism concentrations to be treated have been selected to study bacterial reduction efficacy behaviour as a function of treatment time.

The experimental setup, the sterilizing procedure, and the preparation/analysis of the bacterial reduction efficacy tests will be described in Section 2. In Section 3, results of ozone production, biological tests carried out using different procedures, and the interaction between ozone and cartons containing blisters will be discussed. Finally, the main conclusions and future work will be presented in Section 4.

2 | EXPERIMENTAL AND INSTRUMENTATION SETUPS

In this section, all devices used in the experimental setup, the instruments utilized for its characterization, and cell-culture preparation will be described.

2.1 | Treatment setup

The setup utilized to implement the proposed sterilization method is schematically shown in Figure 1.

A 40 L, 200 bar cylinder contains synthetic air. RONS and charged particles are produced by means of APSJAs operated at atmospheric pressure and contained within a sealed metal box (a detailed description of the plasma reactor will be given in the next subsection). The air flow is controlled by a Bronkhost F-201CV mass flow controller (MFC). The treatment chamber is a $30 \times 30 \times 30$ cubic centimeter aluminum box equipped with a frontal door used to insert and extract samples to be treated. The vacuum inside the treatment chamber is obtained using an Edgard E2M1.5 two-stage rotary pump. The pressure inside the chamber is measured using a Thyracont VD84 compact vacuum meter. All devices shown in Figure 1 are connected to each other by means of vacuum-tight tubes.

The sterilization procedure is performed as follows:

1. The specimen to be sterilized is introduced within the treatment chamber.
2. The pump is switched on and the chamber is evacuated up to an absolute pressure of 5 mbar.
3. The vacuum pump is switched off and the DBD reactor is ignited. Air enriched by RONS and charged particles starts to flow in the treatment chamber at a constant flow rate.
4. When the treatment chamber is filled with the treated air, on reaching the atmospheric pressure condition, the DBD reactor is switched off and the chamber itself is excluded from connecting pipelines by means of needle valves.
5. Active species remain in the chamber for a certain time t_s, needed for bacterial contamination reduction or sterilization.
6. The treatment chamber is evacuated using the pump, removing active species residuals.
7. Fresh air is used to aerate the chamber.
8. The chamber can be opened, and the treated specimen can be removed and handled.

2.2 | Nonthermal plasma reactor and high-voltage power supply system

Reactive species used to obtain the sample sterilization are produced by means of an array of three APSJAs operated at atmospheric pressure air. This pressure level is maintained

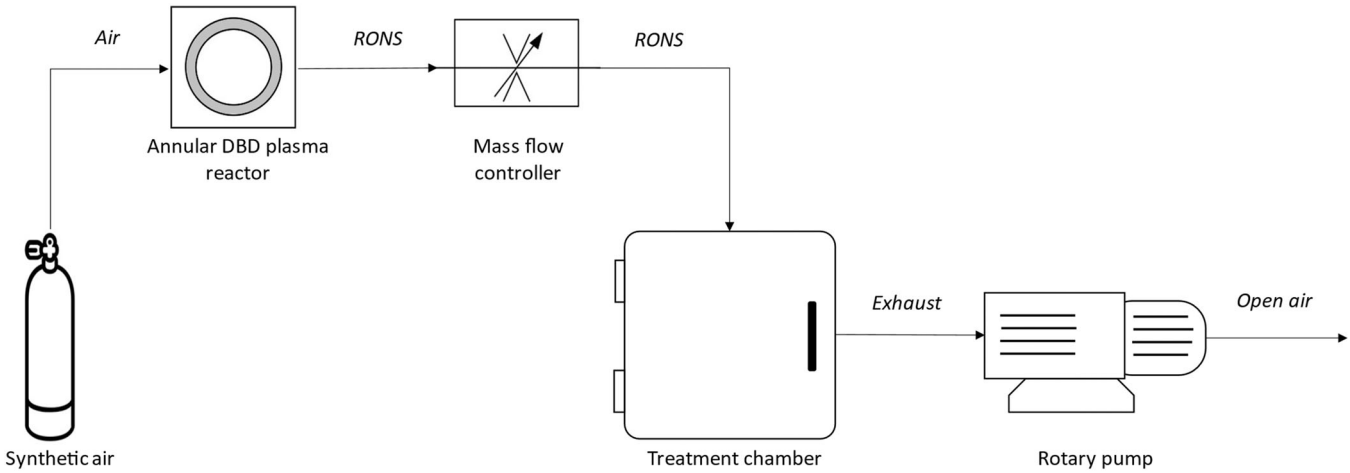


FIGURE 1 Experimental setup used to implement the sterilization method.

by means of the flow controller positioned downstream of the plasma reactor (see Figure 1). Its purpose is to prevent the low pressure in the vacuum chamber from interfering with the pressure of the reactor, which operates at atmospheric pressure during the whole treatment.

A sketch of a single APSJA is shown in Figure 2. The upper exposed electrode is a copper ring with an inner diameter of 30 mm and an outer diameter of 36 mm. This electrode has been obtained using a copper-based paint and it is connected to the high-voltage source terminal. A Macor ceramic slab of $50 \times 50 \times 1\ \text{mm}^3$ has been used as the dielectric material. The lower electrode covers the whole bottom part of the Macor slab, and it is connected to the ground reference terminal. The APSJA used in this work produces both an induced jet normal to the dielectric slab[24] and tangential jets[25] propagating radially starting from the outer perimeter of the annular exposed electrode. These jets enhance the mixing of produced RONS within the incoming air flow.

Typical flow speeds produced by these kinds of actuators are of a few m/s.[4] This speed can be compared to one of the incoming synthetic air. Assuming that the air is incompressible, one has that $Q = Av$, where Q is the flow rate, A is the cross section of the reactor, and v is the gas speed. Assuming $Q = 6\text{L/min}$ and a reactor cross section of $15\text{cm} \times 5\text{cm} = 75\text{cm}^2$, one obtains $v_{\text{AIR}} \sim 1.3\text{cm/s}$. The flow velocity induced by the actuator is thus considerably larger than the one due to the inflow of synthetic air. This leads us to assume that nonnegligible mixing effects are caused by the plasma actuator during the residence time of the gas within the reactor.

Another physical effect produced by the aforementioned jets created by the discharge is advection of the ions produced in the discharge, eventually promoting the sterilization process.[26]

However, since in the experimental setup considered for this work the charged particles are produced at a nonnegligible distance from the treaded specimen, the contribution of ions to the sterilization process is probably limited in this scenario compared to biological effects due to neutral active species.

The three APSJAs used in this work are shown in Figure 3. In the picture, the surface discharge is clearly visible both inside and outside the ring electrode.

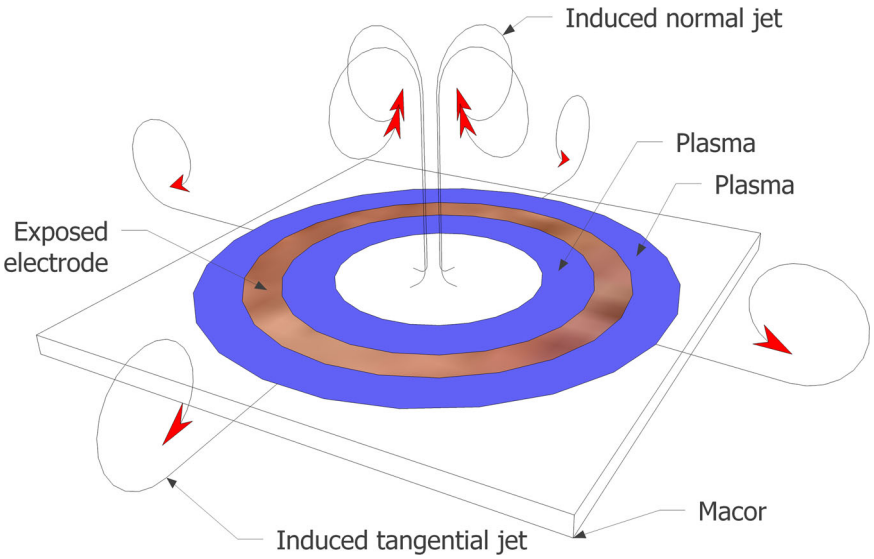


FIGURE 2 Annular plasma synthetic jet actuators (APSJAs) sketch.

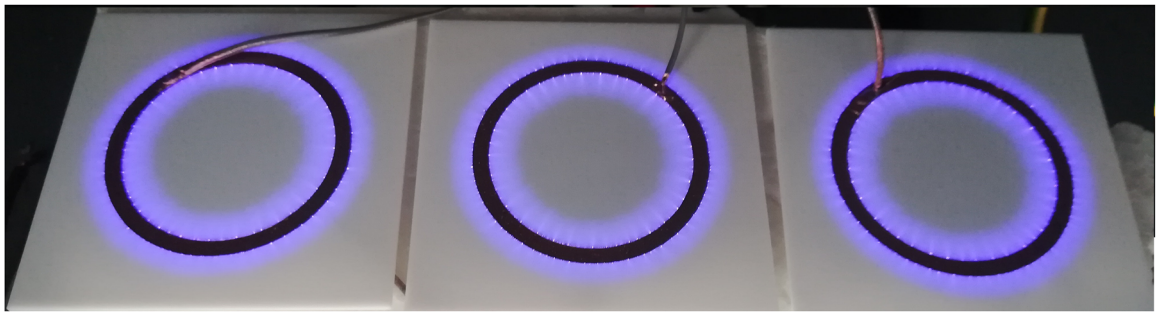


FIGURE 3 Image of the three annular plasma synthetic jet actuators (APSJAs).

To prevent both thermal damage of the dielectric and an increase in the ozone half-life time, a water-cooling system has been built, as shown in Figure 4. The three actuators have been positioned onto an aluminum water-cooled slab and fixed using a Teflon holder with three circular holes necessary to guarantee the propagation of the induced jets. All these devices are contained within a sealed metallic box equipped with air, electrical, and water feedthroughs. The assembly of the three APSJAs, the water-cooling system, and the sealed metal box will be referred as the "reactor" in the following.

The nonthermal plasma reactor has been powered by the high-voltage generator described in reference.[27] A sinusoidal voltage waveform of a 7 kV peak and 16 kHz has been selected to supply the load. The power supply was driven using the duty cycle control strategy, alternatively supplying the discharge with ON/OFF cycles.[28] The duty cycle is defined as:

$$\text{Duty cycle} = \frac{T_{\text{ON}}}{T_{\text{ON}} + T_{\text{OFF}}} \cdot 100\% = \frac{T_{ON}}{T} \cdot 100\%, \quad (1)$$

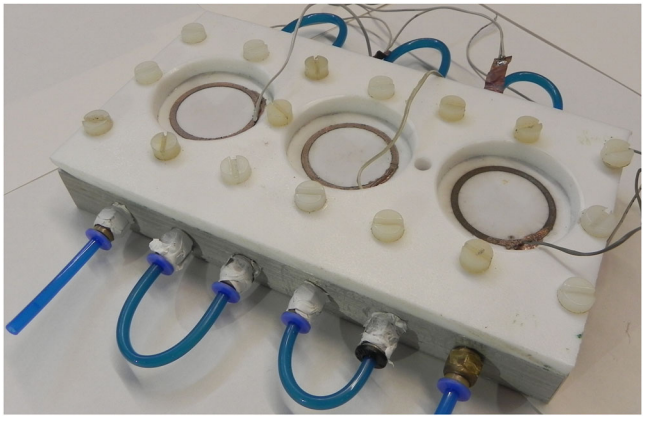


FIGURE 4 Water-cooling system for annular plasma synthetic jet actuators (APSJAs).

where T represents the duty cycle period, T_{ON} is the time interval in which the discharge is ignited, and T_{OFF} is the time interval in which the discharge is turned off. Different duty cycles were tested to maximize the ozone concentration in the treatment chamber.

The average power feeding the reactor was evaluated using Lissajous figures[29] and it was found to be 50 ± 2 W (without a duty cycle control strategy). A Tektronix P6015 capacitively compensated high-voltage probe with a bandwidth of up to 75 MHz was used to measure the high-voltage signal. The charge transferred to the discharge was evaluated by measuring the voltage drop across a 220 nF capacitor using a Yokogawa low-voltage probe with 75 MHz bandwidth. Both signals have been acquired using a Yokogawa DL1740 4-channel, 500 MHz bandwidth, and 1 GS/s oscilloscope.

2.3 | Ozone detector

Ozone is a long-life active species with high oxidative power and subsequently with high inactivation properties useful in indirect sterilization treatments. On the other hand, this molecule is unstable and can be easily destroyed at higher temperatures (at 200°C, its half-life is of a few seconds). This means that—if needed—this compound can be converted into harmless oxygen molecules after the treatment process by increasing the temperature.

The ozone concentration within the treatment chamber was evaluated using the UV absorption method based on the Beer–Lambert law.[30] A sketch of the ozone detector is shown in Figure 5. The sealed UV emitter includes a plastic case, an Optan-255J-BLUV led emitting at 255 nm, and a quartz window transparent to UV radiation. The sealed UV receiver consists of a Hamamatsu S12742-254 photodiode centered at 254 nm, a quartz window, and a plastic case. The emitter and the receiver

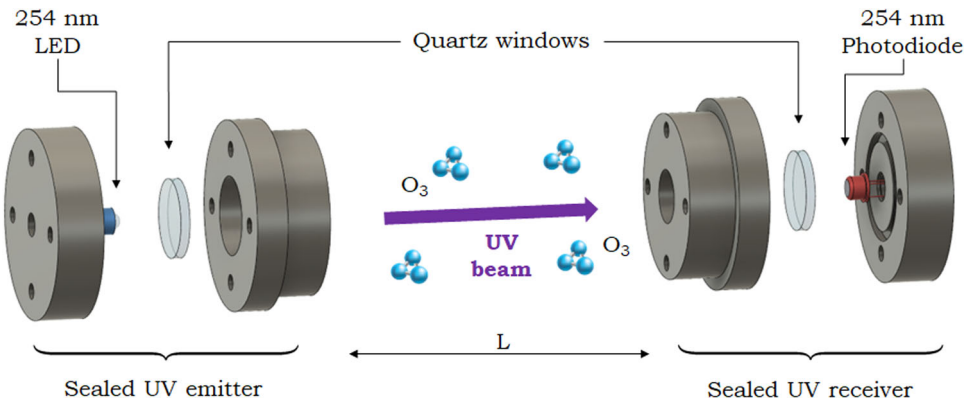


FIGURE 5 Ozone detector scheme.

have been positioned within the treatment chamber at a distance (L in Figure 5) of 5 cm.

2.4 | Bacterial reduction efficacy assays

The bactericidal activity of the active species produced by the plasma reactor was preliminarily tested by exposing specific amounts of *Staphylococcus aureus* ATCC6538P cells (as representative of a pathogenic bacterial species usually associated with hospital-acquired infections) to reactive species in the treatment chamber. The bacterial population to be tested was obtained by inoculating one single colony (from an overnight grown culture on Lysogeny broth [LB] agar plates streaked from the cell stock) of *S. aureus* ATCC6538P in 5 mL of nutrient broth (NB). The culture was grown overnight under shaking at 37°C to reach a final $OD_{600} = 2.5–3$. This culture was then diluted in NB to reach a specific colony-forming unit (CFU) quantity to be tested (e.g., $OD_{600} = 0.01$ corresponded to 10^5 CFU/mL, while $OD_{600} = 0.03$ corresponded to 10^6 CFU/mL) by depositing the suspensions in duplicate on the bottom of a 6-well plate by aliquoting five drops of 20 μL each (a total volume of 100 μL). After this deposition, the 6-well plates were incubated inside a sterile hood without the lid for 1 h to dry the medium, before exposure to the plasma process.

After the plasma treatment, 2 mL of soybean casein digest broth with lecithin and polyoxyethylene sorbitan monooleate (SCDLP) was used to collect the inoculum from the wells. Serial dilutions were performed and inoculated onto LB agar medium to enumerate the bacterial CFUs (after 24 h of growth at 37°C in a static incubator). A 6-well plate control was also inoculated and not exposed to plasma treatment, to evaluate the possible effect of the drying procedure on cell viability.

The bacterial reduction efficacy (BRE) was calculated as:

$$\mathrm{BRE} = \left(1 - \frac{\log(\text{Treated CFU})}{\log(\text{Control CFU})}\right) \bullet 100\%.$$

A bacterial reduction efficacy of 0% represents a negligible effect of the treatment and 100% represents the complete inactivation/killing of the bacterial pathogen under analysis.

2.5 | Sterilization efficacy assay

The sterilization efficacy of the proposed method has been tested on samples characterized by geometries with increasing complexity. The presence of the blister and the packaging carton and different materials of the devices were also considered.

The treated devices are listed below in order of geometrical complexity:

- Drip tubes (small tubes long 2 cm) made of PVC positioned within well plates.
- Three-way stopcocks packed within blisters. This device includes the following materials: ABS, polyisoprene, polycarbonate, polypropylene, and PVC.
- Three-way stopcocks with 10 cm extension line packed in blisters and carton (Figure 6a).
- Connect set with a 0.2 micron solution filter for Chemo drugs, packed in blisters and cartons (Figure 6b). This device, in addition to the 3-way stopcock, includes the following materials: polycarbonate-silicone, acrylic, and PES.

To perform sterility tests of the drip tubes, the ISO 11737-2:2021 was applied by exposing nonsterile infusion samples (in duplicate) to the reactive species. After the plasma treatment, the tubes were immersed in 5 mL of tryptic soy broth (TSB) inside a 50 mL falcon tube, for an incubation time of 14 days at 30°C. The control was represented by a small tube that was not exposed to the plasma treatment. At the end of the 14 days, the change of turbidity (OD_{600}) was evaluated and compared with that from the control culture.

All other disposable medical devices (packed in blisters) were analyzed by an external accredited analysis laboratory following sterility tests described in USP[31] and EU PHARMA[32] reference standards.

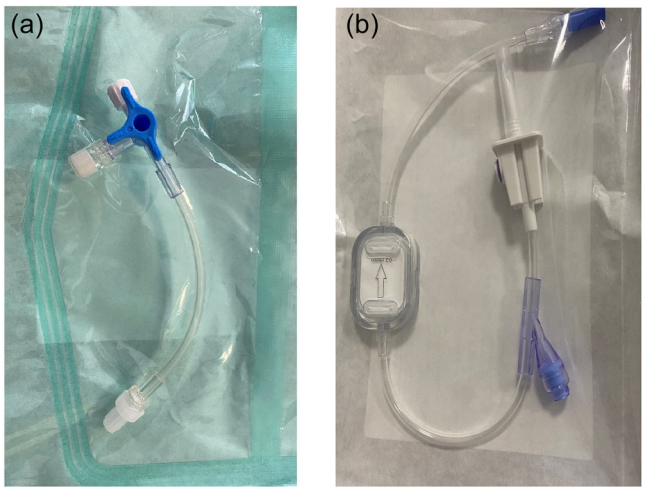


FIGURE 6 Three-way stopcocks with a 10 cm extension line (a) and connect set with a 0.2 micron solution filter for Chemo drugs (b) packed within protective blisters.

3 | RESULTS AND DISCUSSION

In this Section, the operating conditions of the whole setup will be described, with a particular focus on ozone generation. Microbiological tests and interactions between ozone and cartons will be discussed as well.

3.1 | Power supply conditions and ozone measurements

The power supply duty cycle and the mass flow rate have been regulated to maximize the ozone concentration within the treatment chamber. In fact, high ozone levels guarantee good and repeatable inactivation results and, as already reported, ozone is an unstable molecule quite easy to be (thermally) abated after the treatment. As a matter of fact, ozone molecule half life is less than 1 second when temperature exceed 270° (at atmnopheric pressure). The duty cycle was varied from 20% to 100%, with T_{ON} ranging from 5 to 1000 ms. The mass flow rate was varied between 1 and 6 l/min. The value of T_{ON} was found to have negligible impact in the ozone concentration optimization (by keeping the duty cycle unchanged). This parameter was set to 1 s, allowing complete formation and propagation of jets induced by the APSJAs. On the contrary, both the duty cycle and the mass flow rate strongly influence ozone production. The maximum ozone concentration in the treatment chamber was obtained by setting the duty cycle to 60% and the mass flow rate to 3.6 l/min. This supplying configuration leads to an ozone concentration in the treatment chamber equal to 2500 ± 60 ppm.

The evaluation of ozone concentration, together with power consumption and the amount of time needed to fill the treatment chamber, allows evaluation of the ozone yield. This value can be utilized to define the energy consumption of the proposed sterilization method and to perform a comparison with the EtO sterilization process. It is quite difficult to estimate the energy spent for the EtO method, because the energy needed to obtain reagents, catalyst compounds, and the sterilization process itself must be considered. By crosslinking data available in the literature, the energy per unit of the sterilizing agent mass needed for the whole treatment process can be estimated to be around 80 kWh/kg.[33–35] The corresponding energy consumption evaluated for the proposed method, based on the nonthermal plasma indirect treatment, is 29.6 kWh/kg. This value must be increased tacking into account the cooling system energy consumption, synthetic air production, and carton pretreatment. This pretreatment procedure is described in Section 3.4, and it is needed to guarantee sterilization efficacy. A dedicated pretreatment protocol has not been yet defined and the related consumed energy has not been already evaluated.

On the other hand, it must be considered that the energy spent in the DBD reactor produces several reactive species, many of which are N_xO_y.[36,37] These species have been proven to be as useful in the inactivation process as ozone.[38,39] Moreover, synthetic air could be replaced with filtered ambient air (eventually subjected to a dry procedure). For these reasons, energy consumption can be realistically decreased with respect to 29.6 kWh/kg.

Realistically, the overall energy consumption of the proposed method can be considered close to that of EtO-based process. This result contributes to environmental sustainability of the plasma sterilization process.

3.2 | Bacterial reduction efficacy

It is well known that treatment time strongly influences pathogens' reduction efficacy in non-thermal plasma decontamination processes.[40] In this work, the treatment time t_s has been varied between 30 min and 14 h with a control CFU in the range $2 \times 10^4 \div 2 \times 10^5$. Biological tests showed a repeatable minimum of 3 log CFU reduction at the selected shortest treatment time. The longest treatment time has been chosen according to the ozone curve depletion measured within the sealed treatment chamber, shown in Figure 7. In fact, the exponentially decaying curve points out that complete ozone depletion occurs after 14 h. Complete sterilization (BRE = 100%), supported by repeatable results, has been obtained for treatment times longer than 2 h.

European Pharmacopeia standards claim that a decontamination process can be referred as a "sterilization process" only if a reliable Sterility Assurance Level below 10^{-6} is reached (SAL $\leq 10^{-6}$, meaning a minimum of 6 log CFU reduction).[41] Following this regulation, the presented method has been tested for increasing CFU pathogen concentrations of up to 2×10^7. Inactivation efficacy for the longest treatment time (14 h) as a function of the initial control CFU concentration is shown in Figure 8. Data clearly point out that complete bacterial inactivation or killing (BRE = 100%) is achieved for an initial CFU $\leq 6 \times 10^5$. When higher numbers of *S. aureus* cells were used, the bactericidal efficacy decreased, probably due to the bacterial layering during cells' deposition on the well and the subsequent drying procedure. This layering could create a shell that limits/reduces RONS penetration. The protocol adopted in this work for the cells' preparation does not allow to achieve a SAL $\leq 10^{-6}$. In this condition, the proposed new process cannot be referred to as a "certified

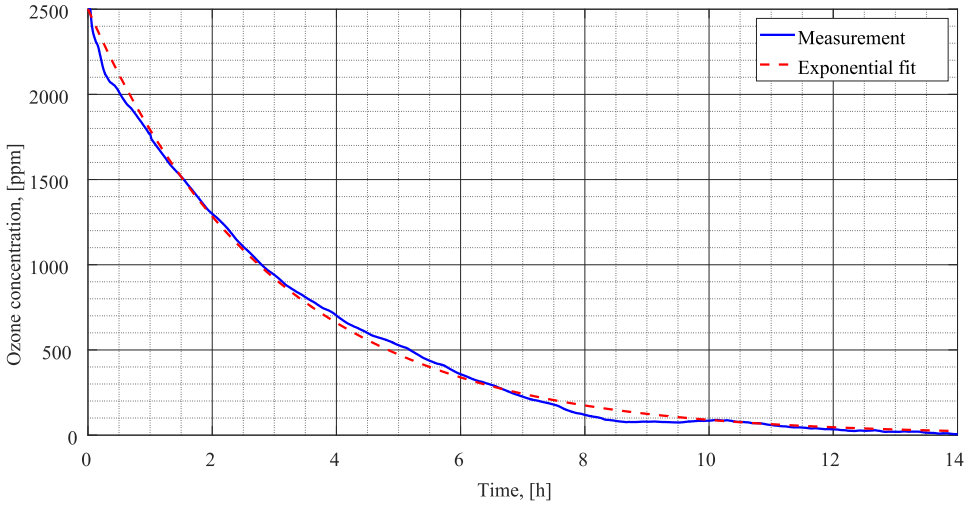


FIGURE 7 Ozone concentration within the treatment chamber as a function of time.

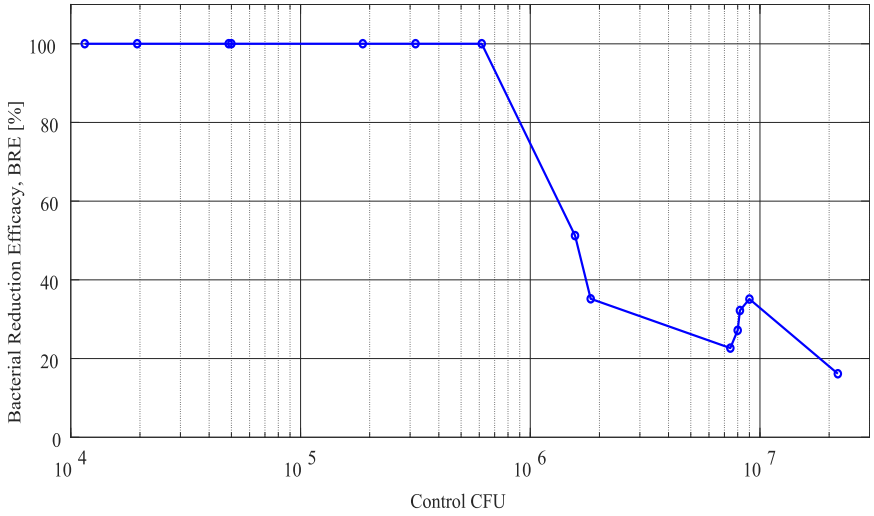


FIGURE 8 Bacterial reduction efficacy obtained with the 14-h treatment as a function of the control colony forming unit (CFU) (the bacterial cells present in the control samples, i.e., the cells recovered from the bottom of a 6-well plate that was processed like the experimental tests, except for the plasma treatment).

sterilization process" following current regulations. A different culture-cell preparation protocol (i.e., without layering) and/or standard biological indicators will be used to verify the treatment efficacy.

On the other hand, the above-mentioned standards (USP and EU PHARMA) do not take into account nonthermal plasma treatments, because these new antimicrobial process typologies have not yet been subjected to a rigid and shared regulatory process. Von Woedke et al.[42] report that single-used medical devices, due to their automated manufacturing procedure, present a low probability of pre-sterilization contamination. For this reason, a SAL of 10^{-4} should be acceptable. They also suggest that "it may be better to call the antimicrobial effects of plasma treatments 'plasma decontamination' or 'plasma antiseptics' as long as a new regulatory sterilization protocol related to plasma treatment will not be made available."[42]

3.3 | Sterilization efficacy assay of disposable devices

The sterilization efficacy of nonsterile infusion drip tubes was assessed based on the standard ISO 11737-2:2020 protocol, which analyzes the change of turbidity (OD600) of the cultural medium (TSB) after immersion of samples. All samples are treated by plasma except for the control. A minimum 2-h treatment guarantees repeatable complete sterilization of all treated tubes (Figure 9).

Disposable devices, under the already mentioned different packed conditions (blister with or without cartons), have been analyzed by an external accredited laboratory following USP and EU PHARMA standard protocols (Section 2.5). These devices have been treated with an exposure time of 2 h. When cartons were used, it has been necessary to pretreat them to enable reactive

species to reach treated devices, assuring sterilization. This procedure is described in the next section.

Sterilization efficacy and accelerated aging tests, based on the ASTM F1980 protocol, have been carried out by the accredited analysis laboratory for packed disposable devices. Results showed that complete sterilization was achieved and maintained during the ageing tests prescribed by the ASTM F1980 protocol. The integrity of packaging and samples was also preserved, suggesting the possibility to use this proposed sterilization method for industrial applications.

3.4 | Carton pretreatment procedure

In the main sterilization processes, samples are packed within their protective blister and stored inside cartons. These boxes are usually contained in pallets ready for the inactivation process and subsequent transportation to customers. Acknowledging the industrial need for backward compatible sterilization processes with respect to production, storage, and shipping chain, in this section, the interaction between cartons and ozone will be reported and discussed.

Carton is made of cellulose, an organic molecule that interacts with ozone,[43] strongly limiting the amount of these reactive molecules responsible for the sterilization process, reaching the treated device. To assess the way in which carton cellulose can decrease the ozone concentration, a carton for commercial purposes has been introduced within the treatment chamber and subjected to the treatment process reported in Section 2.1. The carton has been left inside the chamber, being exposed to reactive species, for 1 h. This procedure will be referred to as the pretreatment cycle and it has been repeated several times with the same carton, evaluating the ozone concentration at the beginning of each 1-h pretreatment. For these tests, an ozone sensor was positioned inside the closed carton. The measured ozone concentration is shown in Figure 10, where the horizontal black dotted line represents the expected ozone concentration without the carton. Figure 10a shows a pretreatment process of a carton stored in ambient air. Figure 10b shows results related to a pretreatment process of a dried carton.

The first column of Figure 10a represents the first 1-h pretreatment and it can be seen that the ozone concentration is negligible, due to the strong interaction between carton cellulose and ozone molecules. In subsequent pretreatments, the ozone concentration increases up to the 9th treatment, where the maximum

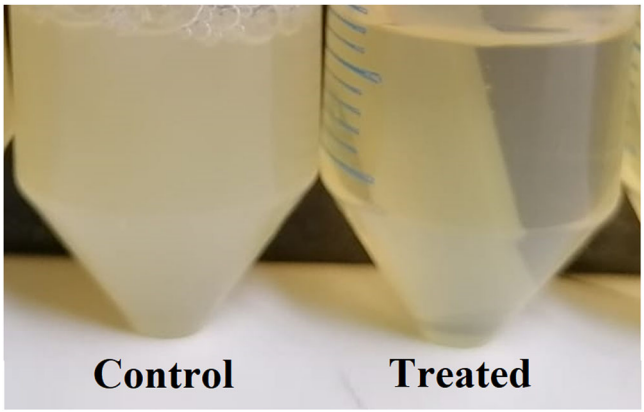


FIGURE 9 Cultural medium turbidity for the control (left-hand side) and treated (right-hand side) samples.

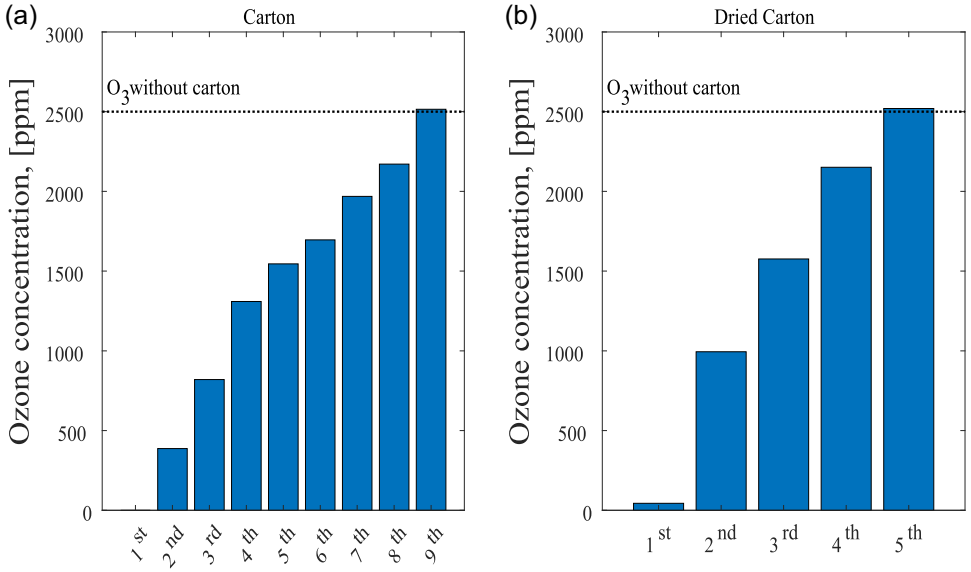


FIGURE 10 Ozone concentration at the beginning of each pretreatment 1-h test. (a) Carton stored in ambient air and (b) dried carton.

ozone concentration is achieved (horizontal black dotted line). To operate with the highest sterilization treatment efficiency, the maximum attainable ozone concentration condition must be reached (2500 ppm). This is because the entire amount of ozone molecules produced by the reactor can be exploited in the sterilization process, limiting its interaction with carton cellulose. Taking this into consideration, another series of pretreatments have been carried out by using a carton previously dried in a controlled oven for 72 h at a temperature of 60°C. A drying procedure has been performed to lower the humidity percentage of the carton. This enables interaction of a higher quantity of ozone molecules with carton cellulose instead of water vapor, thus accelerating the carton's oxidation process. The number of pretreatment cycles needed to reach the desired ozone concentration has been compared between the two different carton conditions (ambient air and dried). The results of the dried carton tests are shown in Figure 10b. Data clearly show that the performed drying procedure halves the number of pretreatments necessary to reach the optimal concentration compared to Figure 10a.

Once the pretreatment procedure has been completed (9th and 5th hour, ambient, and dried carton condition, respectively), the ozone decay over time has been measured. Since the ozone decay trend obtained for both carton conditions is similar, only the ambient results have been considered and discussed in the following. Figure 11 compares these results (red dashed line) with the ozone decay trend in the absence of the carton (blue line), previously shown in Figure 7. The expected outcome was a similar behavior for the two conditions, considering that the initial ozone concentration for sterilization was the same. Conversely, the half-time constant of the red curve is almost halved with respect to the blue one, indicating that an interaction between ozone molecules and carton cellulose was still present. This behavior can be attributed to incomplete carton oxidation after the pretreatment procedures. Regardless of this faster decaying time, disposable devices packed within pretreated cartons (see Section 3.3) were completely sterilized. The success of the sterilization process was probably due to the pretreatment procedure inhibiting interaction between ozone and blister cellulose. In this way a larger concentration of ozone participates to the sterilization process.

The persistence of the pretreatment effect has been investigated as well. Pretreated cartons were stored for 1 week in open air and subsequently subjected to a pretreatment cycle. The initial measured ozone concentration was similar to that obtained during the previous pretreatment (2480 and 2510 ppm, respectively). These results suggest that the achieved pretreatment properties remain unchanged for at least 1 week of storage. As previously reported, complete sterilization of all packed disposable devices inside cartons is guaranteed by the pretreatment process. This demonstrates the feasibility of the proposed sterilization method for industrial applications and its backward compatibility with respect to the ones commonly used nowadays. At this point, it is worth highlighting that the reported results cannot be generalized to all kinds of experimental setups involving cardboard. To the best of the authors' knowledge, there is indeed no scientific literature dedicated to the interaction between cardboard/blisters and ozone. Providing physical information with general validity on the interaction between O_3 and cardboard is a difficult task. This is mainly because no standard cardboard exists, and therefore its specific geometry can considerably vary from one kind of cardboard to the other.

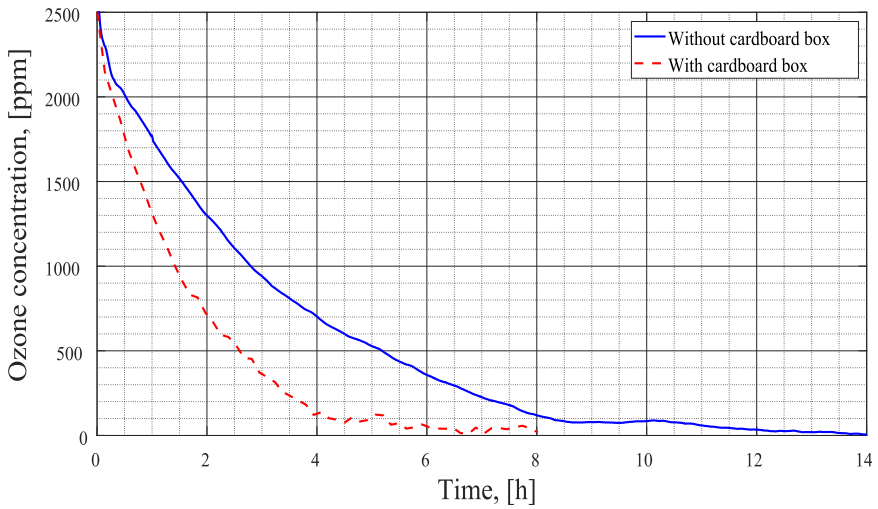


FIGURE 11 Ozone concentration within the treatment chamber as a function of time without the presence of a cardboard box (blue continuous line) and with a cardboard box for "Test 9" and "Dried 5" (red dashed line).

4 | CONCLUSIONS

In this work, an alternative sterilization method for disposable medical devices, based on an indirect non-thermal plasma treatment, has been investigated. A reactor made of an APSJAs array has been used as a plasma source. This kind of actuator produces RONS and charged particles able to significantly reduce the bacterial contamination of surfaces/devices without thermal effects. Preliminary microbiological analyses by exposing a specific *S. aureus* CFU number to plasma RONS were performed. Results showed that a 2-h treatment is sufficient to obtain complete and reliable bacterial killing/inactivation when CFU $\leq 6 \times 10^5$. For higher cell amounts, only a partial bacterial reduction was achieved, even after the longest 14-h treatment time. These results might be attributed to the formation of layers occurring during the bacteria deposition and drying phases, exerting a shielding effect for RONS penetration.

Sterilization efficacy of the proposed method was tested by treating disposable medical devices characterized by geometries of increasing complexity. In addition, the presence of a blister, a carton package, and different materials of the tested devices was also considered. A minimum treatment of 2 h guarantees complete sterilization of all considered samples, even for a complex device such as the "connect set with 0.2 μm solution filter for Chemo drugs," sealed inside the blister and contained in a carton. Accelerated aging tests demonstrated that both sterilization and the integrity of blisters and samples were maintained.

The interaction between ozone and carton package has also been investigated. Cartons strongly interact with ozone, decreasing its concentration within the treatment chamber, limiting the microorganisms' abatement efficacy. To avoid this drawback, a carton pretreatment is necessary to achieve complete sterilization of carton packed devices. After several pretreatment cycles, carried out by a RONS injection into the treatment chamber, the interactions between ozone molecules and carton cellulose decrease, until the initial ozone concentration expected in the absence of the carton package is reached. Once this condition is met, the carton is regarded as "pretreated" and this state is considered as the initial condition for the sterilization process. Pretreated boxes maintain their aquired physico-chemical propreties for at least 1 week of storage in ambient air. The number of pretreatment cycles needed to reach the initial condition for the sterilization process can be decreased by decreasing the carton moisture level.

Overall, the discussed results demonstrate that the sterilization of disposable medical devices can be achieved by using the presented method. The main advantages are limited costs, avoidance in the use and storage of harmful chemical compounds, and backward compatibility in terms of production, sterilization, and shipping chain. Moreover, an estimation of the energy consumption indicates that this method is comparable with respect to the existing EtO-based processes. Furthermore, the presented methodology is environmentally friendly, since it allows to avoid the ecological drawbacks of EtO.

Further work must be done to overcome the limit of inactivation for an initial concentration of 6×10^5 CFU, reaching at least 1×10^6 CFU. Complete inactivation obtained in this condition would yield a SAL $\leq 10^{-6}$, allowing the method to be classified as a "sterilization process" under current regulations. A different culture-cell preparation (i.e., without layering), standard biological markers, and multiple treatment cycles (i.e., increasing RONS quantity taking place in the process) will be considered in future work. A dedicated pretreatment procedure, that is, considering a continuous flow of RONS, will be carried out to accelerate carton pretreatment. Finally, a more accurate analysis of RONS produced by the reactor will be performed and correlated with new biological tests.

ACKNOWLEDGMENTS

This work was supported by the "Proof of Concept Project" funded by the Italian Ministry of Economic Development and by the company A.D.M. srl. Open Access Funding provided by Universita degli Studi di Bologna within the CRUI-CARE Agreement.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Gabriele Neretti http://orcid.org/0000-0003-1059-5733

REFERENCES

[1] Region and Segment Forecasts from 2021 To 2028', Report ID: MN17617669, **2021**.

[2] Gamma Industry Processing Alliance, 'A Comparison of Gamma, E-beam, X-ray and Ethylene Oxide Technologies for the Industrial Sterilization' of Medical Devices and Healthcare Products, **2017**.

[3] United States Department of Labor. Occupational Safety and Healthcare Administration, Ethylene Oxide. https://www.osha.gov/ethylene-oxide

[4] G. Neretti, P. Seri, M. Taglioli, A. Shaw, F. Iza, C. A. Borghi, *J. Phys. D Appl. Phys.* **2016**, *50*(1), 015210.

[5] X. Xu, *Thin Solid Films* **2001**, *390*(1-2), 237.

[6] U. Kogelschatz, *Plasma Chem. Plasma Process.* **2003**, *23*(1), 1.

[7] A. A. Fridman, G. G. Friedman, *Plasma Medicine*, John Wiley & Sons, Chichester **2013**.
[8] M. Laroussi, *Plasma* **2018**, *1*(1), 47.
[9] G. Colonna, C. D. Pintassilgo, F. Pegoraro, A. Cristofolini, A. Popoli, G. Neretti, A. Gicquel, O. Duigou, T. Bieber, K. Hassouni, L. Laguardia, *Eur. Phys. J.* **2021**, *75*(6), 183.
[10] O. O. Olatunde, S. Benjakul, K. Vongkamjan, *J. Food Safety* **2019**, *39*(6), e12705.
[11] G. Neretti, M. Taglioli, G. Colonna, C. A. Borghi, *Plasma Sources Sci. Technol.* **2016**, *26*(1), 015013.
[12] J. W. Lackmann, J. E. Bandow, *Appl. Microbiol. Biotechnol.* **2014**, *98*(14), 6205.
[13] A. Filipić, I. Gutierrez-Aguirre, G. Primc, M. Mozetič, D. Dobnik, *Trends Biotechnol.* **2020**, *38*(11), 1278.
[14] Y. H. Ryu, Y. H. Kim, J. Y. Lee, G. B. Shim, H. S. Uhm, G. Park, E. H. Choi, *PLoS One* **2013**, *8*(6), e66231.
[15] G. Neretti, B. Morandi, M. Taglioli, G. Poglayen, R. Galuppi, G. Tosi, C. A. Borghi, *Plasma Med.* **2018**, *8*(2), 155.
[16] N. N. Misra, B. Yadav, M. S. Roopesh, C. Jo, *Compr. Rev. Food Sci. Food Saf.* **2019**, *18*(1), 106.
[17] K. H. Becker, U. Kogelschatz, K. H. Schoenbach, R. J. Barker, *Non-Equilibrium Air Plasmas at Atmospheric Pressure*, CRC Press, Boca Raton, FL, **2004**.
[18] L. Zhao, K. Adamiak, *Part. Sci. Technol.* **2016**, *34*(1), 63.
[19] N. Benard, E. Moreau, *in 6th AIAA Flow Control Conf.* New Orleans (LA), USA, 25-28 June, **2012**.
[20] C. A. Borghi, *in 37th AIAA Plasmadyn. Lasers Conf.* San Francisco (CA), USA, 5-8 June, **2006**.
[21] M. Taglioli, A. Shaw, A. Wright, B. FitzPatrick, G. Neretti, P. Seri, C. A. Borghi, F. Iza, *Plasma Sources Sci. Technol.* **2016**, *25*(6), 06LT01.
[22] G. Neretti, A. C. Ricchiuto, C. A. Borghi, *J. Phys. D Appl. Phys.* **2018**, *51*(32), 324004.
[23] G. C. C. Mendes, T. R. S. Brandão, C. L. M. Silva, *Am. J. Infect. Control* **2007**, *35*(9), 574.
[24] A. C. Ricchiuto, C. A. Borghi, A. Cristofolini, G. Neretti, *Plasma Process. Polym.* **2021**, *18*(4), 2000214.
[25] A. Cristofolini, G. Neretti, F. Roveda, C. A. Borghi, *J. Appl. Phys.* **2012**, *111*(3), 033302.
[26] G. Neretti, A. C. Ricchiuto, R. Galuppi, G. Poglayen, B. Morandi, E. Marotta, C. Paradisi, F. Tampieri, C. A. Borghi, *Plasma Med.* **2018**, *8*(3), 255.
[27] G. Neretti, A. Popoli, S. G. Scaltriti, A. Cristofolini, *Electronics* **2022**, *11*(10), 1536.
[28] G. Neretti, M. Ricco, *Electronics* **2019**, *8*(10), 1137.
[29] M. Hołub, *Int. J. Appl. Electromagnet. Mech.* **2012**, *39*(1-4), 81.
[30] S. O'Keeffe, *Journal of Physics: Conference Series*, IOP Publishing, Medway (Kent), UK, **2005**.
[31] USP Ed.38: **2015** "Sterility tests" Par. 71, 72-75.
[32] EU PHARMA Ed. 8.2:**2014**, par. 2.6.1, pp. 175-178.
[33] A. Ghannadzadeh, M. Sadeqzadeh, *Clean Technol. Environ. Policy* **2017**, *19*(8), 2145.
[34] S. Rebsdat, D. Mayer, *Ullmann's Encycl. Indust. Chem.* **2000**, *13*, 457.
[35] D. Ozokwelu, J. Porcelli, P. Akinjiola, *Office Energy Efficiency Renew. Energy US Dep. Energy* **2006**, 1.
[36] S. Park, W. Choe, C. Jo, *Chem. Eng. J.* **2018**, *352*, 1014.
[37] Y. Sakiyama, D. B. Graves, H. W. Chang, T. Shimizu, G. E. Morfill, *J. Phys. D Appl. Phys.* **2012**, *45*(42), 425201.
[38] M. J. Pavlovich, T. Ono, C. Galleher, B. Curtis, D. S. Clark, Z. Machala, D. B. Graves, *J. Phys. D Appl. Phys.* **2014**, *47*(50), 505202.
[39] M. Shomali, D. Opie, T. Avasthi, A. Trilling, *PLoS One* **2015**, *10*(6), e0130043.
[40] A. Sakudo, Y. Yagyu, T. Onodera, *Int. J. Mol. Sci.* **2019**, *20*(20), 5216.
[41] Guideline on the sterilisation of the medicinal product, active substance, excipient and primary container, edited by the European Medicines Agency, EMA/CHMP/CVMP/QWP/850374/2015, **2019**.
[42] T. Von Woedtke, A. Kramer, K. D. Weltmann, *Plasma Process. Polym.* **2008**, *5*(6), 534.
[43] E. Montet. Investigation of the consequences of the use of ozone in the bleaching of cellulosic fibres. Diss. Université Grenoble Alpes, **2021**.

How to cite this article: P. Seri, S. Nici, M. Cappelletti, S. G. Scaltriti, A. Popoli, A. Cristofolini, G. Neretti, *Plasma Processes Polym.* **2023**;*20*:e2300012. https://doi.org/10.1002/ppap.202300012