Surface Sterilization of Medical Low Density Poly(ethylene) films by Dielectric Barrier Discharges

L. Benterrouche[1], S. Sahli[1], N. Kacem Chaouch[2], M. T. Benabbas[1], S. Benhassine[2], M. Kara Ali[2]

[1] *University of Frères Mentouri, Laboratory of Microsystems and Instrumentation (LMI), Electronic department, Faculty of Technologies Sciences, Constantine-Algeria*

[2] *University of Frères Mentouri, Laboratory of Mycology, Biotechnology and Microbial Activity (LaMyBAM), Microbiology department, Nature and Life Sciences Faculty, Constantine-Algeria*

A cold plasma was generated by Dielectric Barrier Discharges (DBD) for the sterilization of medical low density poly(ethylene) (LDPE) films contaminated by E. coli bacteria. The plasma was created in atmospheric air inside a controlled chamber using a pulsed high voltage power supply. The inactivation efficacy of the created plasma was investigated as a function of plasma treatment time and plasma treatment mode (direct or remote plasma mode) using optical microscopy observations and loss mass measurements. Moreover, the surface effect of the LDPE films on the sterilization efficacy was investigated. When the films are treated in the DBD plasma mode, about 98% of the bacterial cadavers' have been removed in about 15 min on LDPE contaminated films and more earlier on plasma pre-treated LDPE contaminated films. When the contaminated films are treated in the remote plasma mode (remote plasma), only about 90% of bacteria cadavers' are removed.

1. Introduction

The introduction of heat-sensitive polymer materials in almost all medical tools has raised the necessity of developing new sterilization processes for this kind of polymers. Atmospheric pressure plasma discharges have the ability to generate at ambient conditions from different working gases reactive spaces that may participate actively in the inactivation of microorganisms [1]. This makes these kind of plasmas a promising field for the sterilization of heat-sensitive materials such as polymers and plastics [2, 3]. In addition, the use of cold plasmas for sterilizing of such materials does not affect their bulk properties but only some nano-layers near the surface [4]. Cold plasma sterilization is now considered as a safely methods compared to conventional sterilization methods, which work at low temperatures but use dangerous sterilizing agents for the user and/or the patient health such as gamma radiation and ethylene oxide gas. Furthermore, plasmas discharge have sterilization pronounced efficiency on several types of resistant to conventional methods bacteria and especially the pathogen ones [5]. Plasmas using in sterilization are carried out at low pressure in a various gas precursors like argon and oxygen [6] or at atmospheric pressure. However, plasmas created in atmospheric pressure and especially in the air have a more reactive species such as atomic oxygen, atomic nitrogen, ozone, OH, NO ..., which are known to have a great degradation effect of the bacterial structure [7].

In the present study, we have carried out some investigations on the effect of Dielectric Barrier Discharge (DBD) plasma generated in air at atmospheric pressure using a homemade reactor and pulsed high voltage power supply, on the sterilization efficacy of E. coli contaminated medical low density poly (ethylene) (LDPE) films. The influences of the plasma treatment time, the plasma treatment mode and the LDPE films surfaces on the sterilization efficacy have been studied using microscopy observations, loss of mass and contact angle measurements.

2. Experimental

2.1. Description of the experiments

The experimental apparatus used in this study is represented on figure 1. It consists of two plane-parallel metallic electrodes of 80 mm in diameter spaced by a gap varying from 1 to 5 mm. Our samples were placed on the grounded electrode (lower electrode). The upper electrode was covered with a glass disk of 110 mm of diameter and 1.3 mm of thickness and was connected to a homemade-pulsed high voltage power supply to generate the DBD plasma in the atmospheric air gap.

The DBD reactor was installed in a homemade test chamber with the dimensions of 140 mm (width) x 140 mm (depth) x 100 mm (height).

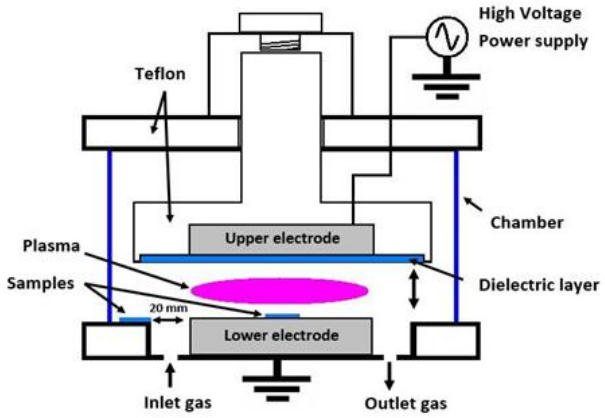


Figure 1. Schematic diagram of the experimental setup.

2.2. Method

Transparent low density poly(ethylene) (LDPE) films of 50 μm in thickness were used in this study. These films were cut in pieces of dimensions 20 x 20 mm^2; each one was washed successively in Bleach, methanol and twice distilled water and then dried naturally at room temperature. An air atmospheric DBD plasma discharge was then used to treat during 5 min a few of these washed substrates (pre-treated samples). 100 μl of E. coli bacteria culture was transferred and spread out on each untreated or pre-treated LDPE surface substrates and then, dried at room temperature for 1 h. For each plasma treatment time, one pre-treated and one untreated contaminated samples are placed in the discharge mode (in this mode, samples are placed on the lower electrode unprotected from the discharge plasma) and another untreated contaminated sample was placed in the remote plasma mode, outside of the plasma discharge by an arbitrary distance of 20 mm (Fig. 1). These three contaminated LDPE samples placed in discharge and remote plasmas modes were exposed during different time to air DBD plasma created by applying a high voltage of 9 kV and a frequency of 0.2 kHz between the reactor two electrodes distant of 3 mm.

2.3. Contact Angle Measurements

The surface wettability of the treated films and its evolution as function of treatment time was characterized by contact angle technique at 26°C and 42% RH of relative humidity. A distilled water drop was delivered by a micro syringe onto the films surface immediately after plasma treatment experiments. To lessen the effect of gravity, the volume for each drop was regulated to 5 μl. The contact angles were measured at least three different locations on the treated samples and a maximum error less than ± 2° had been recorded.

2.4. Mass loss measurements

To estimate any effects on the mass of the E. coli contaminated layers spread out on the LDPE film surface, the weight of each sample was measured before and immediately after the plasma treatment, using a microbalance (Adventurer OHAUS, AR0640). By designing M_0 as the weight of the sample before plasma treatment and M_t its weight after the plasma treatment, the mass loss induced by the DBD plasma treatment is described by the weight loss and can be calculated using the following expression:

$$\text{Mass Loss} = (M_0 - M_t) / M_0 \quad [100\%] \qquad (1)$$

3. Results

In this study, Escherichia coli bacteria was used as biological indicator representing a Gram-negative bacteria commonly used as the reference microorganisms in the development of new sterilization processes. Before any DBD plasma treatment, the optical microscope photograph of figure 2 shows a high E. coli bacteria concentration on the surface of the transparent medical low-density poly(ethylene) (LDPE) substrate film.

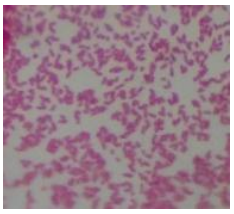
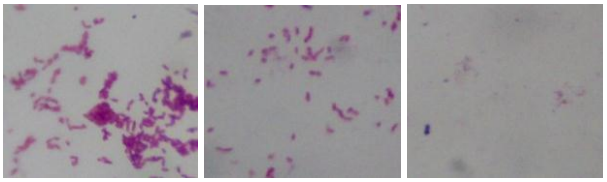


Figure 2. Optical microscope photograph (1000x magnification) of E. coli before plasma treatments.

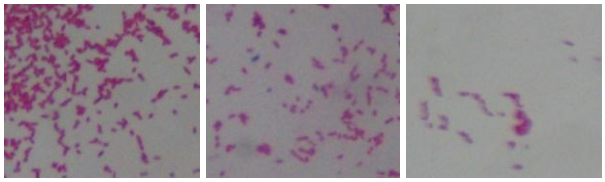
This bacterial concentration decreases significantly with increasing the plasma treatment time. As it is shown on Fig. 3, a quasi-total disappearance of the E. coli cadavers was observed after 15 min on the contaminated untreated substrates treated in the plasma discharge mode. In this case, E. coli bacteria were exposed directly to plasma reactive species (such as hydroxyl radicals (OH), ozone...), to UV radiation and to charged particles. An ablation process of the bacterial cells can explain the disappearance of the bacterial cadavers by these reactive plasma species [8] and/or by a sputtering effect of the bacterial structure

induced by the charged and/or the energetic plasma species [9]. However, as it is represented on Fig. 4, the efficiency of this sterilization effect is less important for samples treated in the remote plasma mode. The concentration of the bacteria cadavers are more important on the contaminated surface films treated in this mode than on those treated in the direct mode. This difference in the sterilization efficacy is due to a less bacteria exposition to the UV radiations, and/or to the charged and energetic plasma species (in the remote plasma, species are less energetic and may be less reactive).



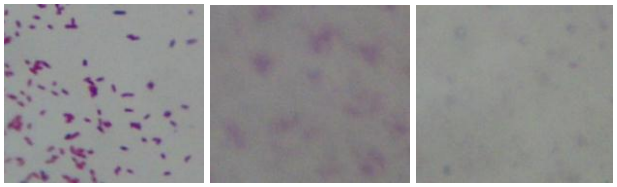
(a) (b) (c)

Figure 3. Optical microscope photographs (1000x magnification) of E. coli after direct plasma treatment during (a) 5 min; (b) 10 min; (c) 15 min.



(a) (b) (c)

Figure 4. Optical microscope photographs (1000x magnification) of E. coli after remote plasma treatment during (a) 5 min; (b) 10 min; (c) 15 min.



(a) (b) (c)

Figure 5. Optical microscope photographs (1000x magnification) of E. coli after direct plasma treatment during (a) 5 min; (b) 10 min; (c) 15 min of pre-treated LDPE films.

In Fig. 5, we have reported the effect of direct DBD plasma treatment on E. coli contaminated LDPE samples previously treated by air atmospheric pressure DBD plasma (pre-treated substrates). Optical microscope photographs showed a significant decrease of the bacterial cadavers' concentration with the increase of plasma treatment time. For only 10 min of direct DBD treatment, no entire E. coli cadaver was observed on the contaminated films substrate. The surface modifications of non-contaminated LDPE substrates induced by the air atmospheric pressure plasma in direct mode has been characterized by contact angle measurements. The contact angle of this plasma pre-treated LDPE substrates decreases significantly with the increase of the plasma treatment time: from 90° for substrate control to 30° for LDPE substrates treated during 15 min (Fig. 6).

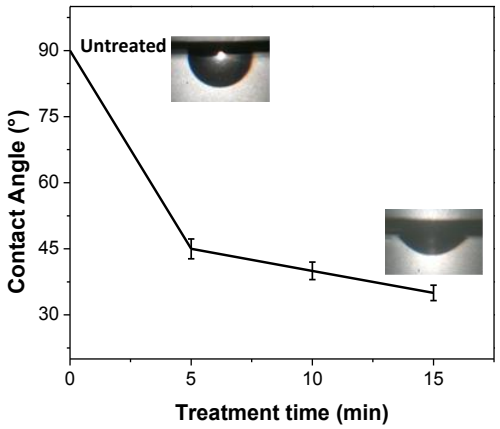


Figure 6. Effect of plasma treatment time in direct plasma mode on the contact angle of the LDPE films.

This behaviour of the contact angle has been explained by the creation on the LDPE surface, of polar groups (such as carbonyl (C = O) and hydroxyl (-OH)) by the charged species of the air plasma [10]. Thus, the high bacteria removal observed on the previously plasma treated LDPE substrates may be linked to an increase of their wettability after their exposition to the air atmospheric pressure DBD plasma. The improved of their hydrophilicity, allowed a better bacteria culture spreading and then, a thinner layer of the bacteria culture was obtained on the surface substrates. The microorganism distribution on the DBD plasma pretreated LDPE surface is then more homogenous than that observed on the untreated substrates. The weak wettability of the untreated substrates leads to an inhomogeneous bacteria distribution and the formation of bacteria clusters-like with thicker thickness than that observed on microorganisms layers spreading on plasma pretreated LDPE films. The formation of these bacteria cluster-like, makes the plasma species penetration difficult and therefore the sterilization process takes more time.

Fig. 7 shows the effect of treatment plasma time on the weight loss of the sterilized LDPE films. It is shown that the E. coli contaminated pre-treated

substrates exposed to direct DBD discharges have a more significant loss of mass compared with the contaminated untreated substrate exposed in direct or remote plasma mode. This behavior is in good agreement with the optical microscopy observations presented on figures 3 and 5 where it appears that the pretreated substrates have the better sterilization effect: a quasi-complete disappearance of bacteria cadavers was obtained in about 10 min for these samples and after more than 15 minutes for untreated substrates.

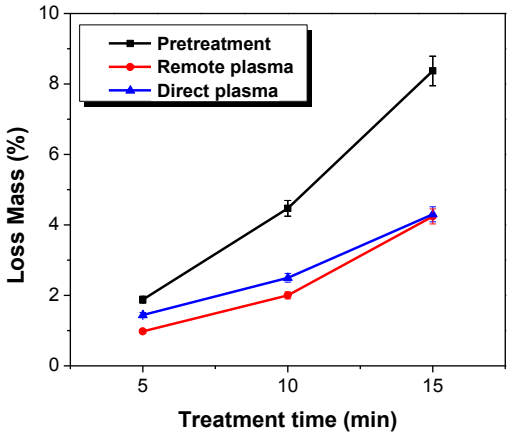


Figure 7. Effect of plasma treatment time on mass loss of E. coli contaminated LDPE films.

4. Conclusion

The sterilization of E. coli bacteria on the surface of low-density poly(ethylene) LDPE films by DBD plasma at atmospheric pressure using air as a discharge gas and the surface modification properties of the sterilized films was investigated. It has been revealed that the plasma treatment time, the plasma treatment mode and the initial state of the substrate have a strong effect on the sterilization efficiency. It was found that the best plasma sterilization efficiency was obtained on plasma pre-treated samples and the less pronounced one was found on samples treated on remote DBD plasma mode.

5. Acknowledgment

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6. References

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