

Use of a Microwave Plasma Process at Atmospheric Pressure for Bacterial Inactivation without Thermal Effects

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Abstract

An atmospheric microwave plasma argon was used for the inactivation of bacteria *E. coli*. The employed device, called Axial Injection Torch (or TIA for Torche à Injection Axiale), consisted of a microwave power source, a waveguide and a gas supply system. Using this argon plasma source, we studied the effects of the exposure time, the exposure distance, the input power, and the gas flow rate on the reduction rate of *Escherichia coli* cells. The first part of the study was carried out with a static sample exposed to the plasma and then in the second part the sample was set in motion relative to the plasma jet. A log reduction number of *E. coli* of 4 (10^{-4} CFU/mL) was obtained with UV and active species, for UV only a log of 1 (10^{-1} CFU/mL) was obtained.

Keywords

Atmospheric Pressure Plasma, Microwave Plasma Torch, Disinfection, Bacteria

1. Introduction

Disinfection is an operation that makes it possible to fight against the proliferation of bacteria and to temporarily eliminate some microorganisms. Traditionally, decontamination methods are based on the use of high temperature or chemical compounds (*i.e.*: chlorine and derivatives). But depending on the field of applications, these traditional sanitation methods can be unsatisfactory because of effluents to be treated or heat sensitive surfaces. Moreover, many studies have demonstrated that traditional sanitation methods cannot completely

eradicate microorganisms from food-processing surfaces [1] [2]. That is why the use of cold atmospheric pressure plasma is of great of interest. It offers rapid antimicrobial action against a broad spectrum of pathogens, while minimally affecting the exposed surface. Furthermore, it is environmentally friendly and requires no consumables except some gas (Ar, O₂ or air) [2]. These plasmas can be produced with different sources like corona discharges, Dielectric Barrier Discharges (DBD, planar or cylindrical configuration) or plasma jets working at different frequencies, radio-frequencies or microwaves [3]-[11]. Plasma torches work in open air or in controlled atmosphere. They are very versatile systems which find applications in a wide variety of fields such as the production of thin layers, surface modification but they are increasingly used for surface decontamination [3].

Inactivation of micro-organisms is believed to be due to species produced in the plasma: mainly by radicals and reactive oxygen and nitrogen species, also called RONS [11]-[16] (OH, O, O₃, NO...) and charged ones (O₂⁻, NO₃⁻...), affecting the cell membranes. UV can also create damages, especially UV C (220 - 280 nm) [12] [14]. The time of exposure influences the efficiency of the treatment [1] [16] [17] and the influence of the process parameters and the specific action of species and UV rays are still under discussion [16] [18] [19] [20] [21].

Escherichia coli abbreviated *E. coli* is commonly used in biological experiments thanks to its ability to reproduce very quickly (every 20 minutes) and to the knowledge accumulated on its genome, its physiological properties and its metabolism. Whatever the means of treatment, bacteria die when cell membranes are broken or when DNA (deoxyribonucleic acid) is damaged [18]. The application of a treatment results in decontamination, disinfection or sterilization depending on the intensity (Table 1). A reduction log of at least 4 or 5 is generally sought, to have a suitable disinfection process.

The plasma source used in this study is TIA (Torche à Injection Axiale), it works with microwaves, in open air and the plasma forming gas is argon. It offers a wide range of working conditions, in terms of microwave power, gas flow rate, distance to the substrate and injection of additional gas or vapour. The TIA has already been used to deposit thin layers of crystallized TiO₂ [22] from TTIP precursor. For this, relatively high microwave power and low working distance allowing to transfer enough energy were selected to get in situ crystallization of TiO₂. The oxygen present in the TiO₂ comes from the ambient air. The power of plasma dissociates molecules (O₂, N₂) from the air to form reactive

Table 1. Correspondence between the number of Log and the percentage of abatement.

Reduction Log	Reduction%
2	99
3	99.9
4	99.99
5	99.999
6	99.9999

species (O^* , N^* , O_3 , ...). So, it can be supposed that these working conditions combining the presence of oxygen and nitrogen reactive species and high temperatures would allow to inactivate bacteria. As these conditions are not satisfactory for the treatment of heat sensitive surfaces, it is necessary to investigate the potential of this process for low energy plasma conditions.

The aim of this paper is to optimize the exposure time, exposure distance, input power, gas flow rate for inactivation of *E. coli* and to study the contribution of the UV on the bacteria disinfection, using the TIA, while avoiding the use thermal effect of the plasma. For this, cells of *E. coli* were exposed to the plasma in different conditions in order to optimize the log reduction number. As the objective of this work is to take advantage of the active species and UV of the plasma, the conditions generating lethal effects on bacteria cells, *i.e.* temperatures higher to 45°C, were rejected. The working conditions were firstly selected for treatments without moving the samples. Then the selected parameters were applied with moving the sample in front of the plasma to get homogeneous treatment. Two complementary exposure conditions were compared namely with and without filtering UV.

2. Experimental Methods

2.1. Culturing Conditions of *E. coli*

- Preparation of bacterial suspension

E. coli CIP 52.172 (CRBIP, French) was maintained on agar (Trypto Casein Soy Agar (TSA), BK047HA, BIODIAGNOSTICS) at 5°C and the culture was grown in Trypto Casein Soy Broth (TSB), BK046HA, BIODIAGNOSTICS at 37°C to get of 10^9 CFU/ml (Colony-Forming Unit/mL). Decimal dilutions were applied in 0.9% physiological saline solution liquid, and then the dilutions were spread on TSA (Figure 1). Each Petri dish was made twice.

These dilutions made possible to determine the concentration of bacterial culture by counting colonies on the Petri dishes. The concentration of bacterial suspension was calculated with the formula from the norm NF EN ISO 8199 [23] (Equation (1)).

$$N = \sum C / V_{tot} \times V_s \quad (1)$$

with:

- N : concentration CFU/mL
- $\sum C$: sum of colonies of all Petri dishes from accounting dilutions
- V_{tot} : total sample volume = $[(n_a \cdot V_a \cdot d_a) + (n_b \cdot V_b \cdot d_b) + \dots]$ (n = number of colonies per Petri dish, V = volume (mL), d = dilution)
- V_s : Reference volume chosen to express the concentration of microorganisms in the sample (mL)
- **Preparation of Petri dishes for plasma treatment**

The initial bacterial suspension was diluted to 10^8 CFU/mL, then 30 μ L of the diluted culture are spread on the agar, as shown in Figure 2.

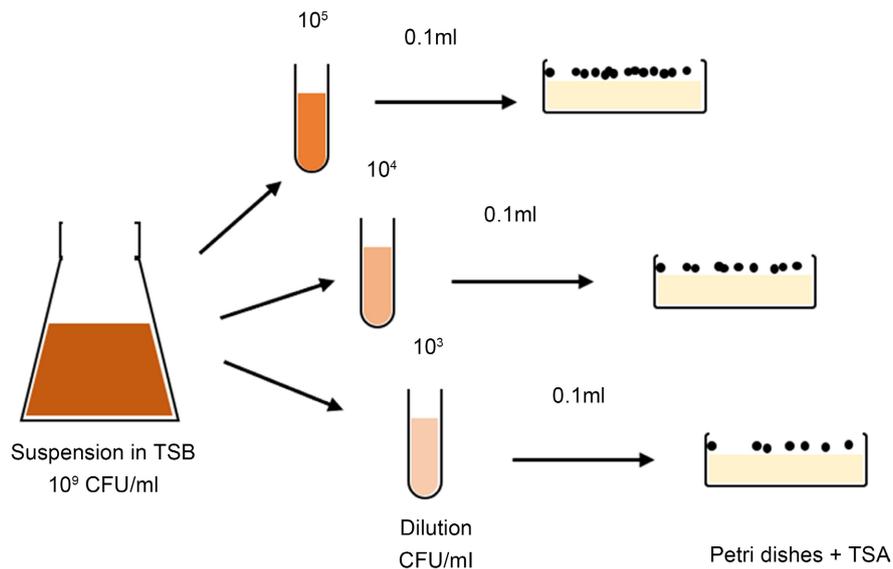


Figure 1. Control of culture concentration by dilution/spreading.

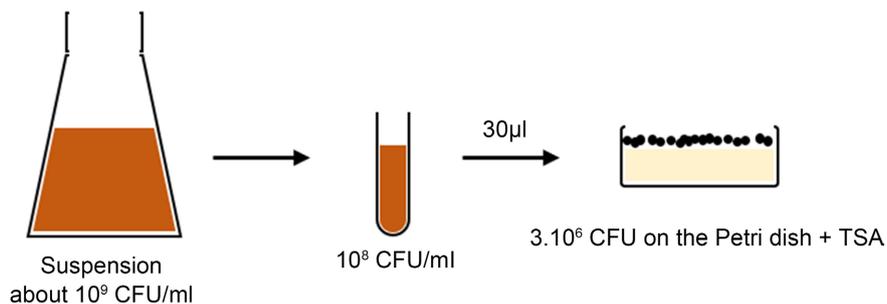


Figure 2. Preparation of the Petri dish with bacterial suspension.

In order to check bacterial growth, Petri dishes not exposed to plasma are made and will be called “controls”. Two controls by dilution were made.

After the plasma treatment, samples and controls were incubated at 37°C during 24 h. The number of colonies which formed on the agar was counted visually and considered as the number of surviving CFU.

- **Determination of the abatement log**

The reduction log represents the ratio of amount deposited (CFU) to survivors able to form colonies (CFU) and is defined according to the Equation (2).

$$\text{Reduction Log} = \text{Log}(\text{initial CFU}/\text{final CFU}) \quad (2)$$

This formula is applicable only when colonies on the samples and controls can be counted visually (<300 CFU).

2.2. Experimental Setup

The atmospheric pressure plasma system used in this study is an axial injection torch (TIA), represented on **Figure 3**. Microwaves are provided by a SAIREM1200 KED generator (2.45 GHz) and transported by a rectangular waveguide to supply the cylindrical outer conductor of the coaxial guide. The input power was varied

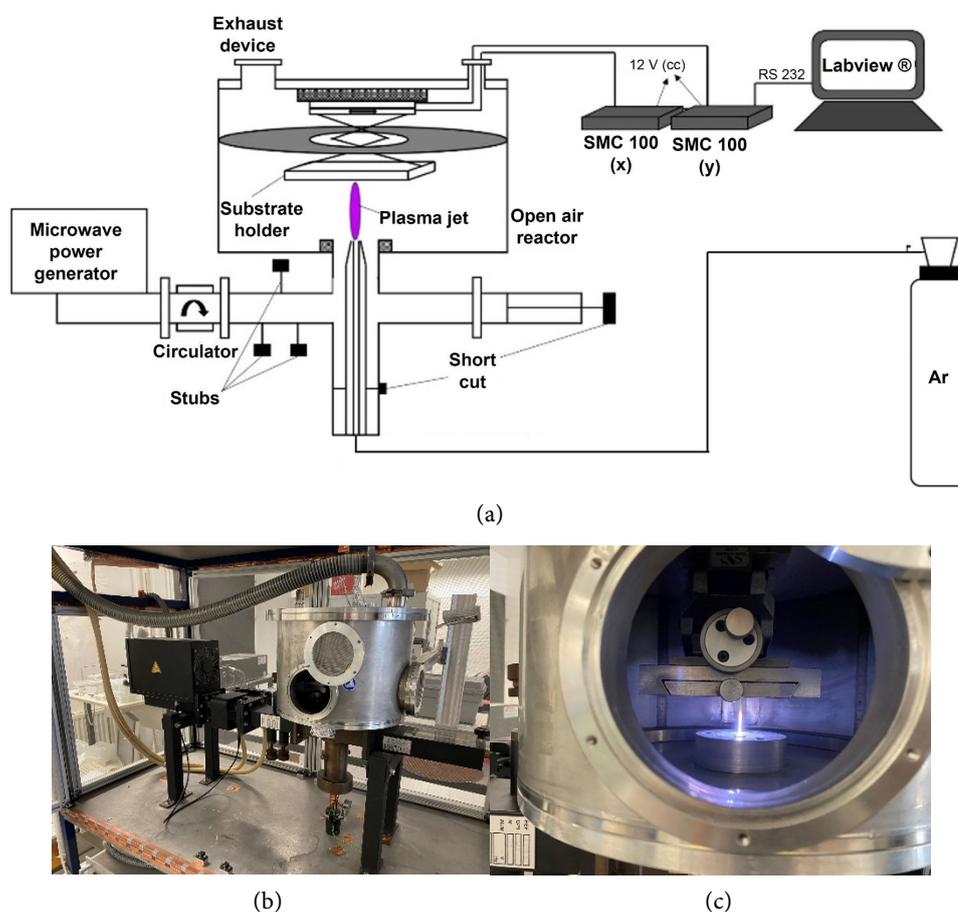


Figure 3. (a) Axial injection torch scheme; (b) photo of the experimental set up; (c) photo of the plasma jet in the open-air reactor.

between 200 and 250 W. The nozzle, with a 2 mm inner diameter, is placed on the top of the coaxial conductor in a large cylindrical open-air reactor. The sample holder faces the plasma jet and can be moved along the x and y-axis using a Labview[®] program. Its height can be changed manually. The exposure distance between the nozzle exit and the sample was set between $z = 30$ mm and $z = 35$ mm. An exhaust device removes gas produced during exposition. Argon (Air-Liquide Alphagaz I, purity > 99.999%) was used as plasma gas with flow rates of 3, 5, 7 and 10 slpm.

The trajectory of the plasma jet on the Petri dishes during the treatment is described on **Figure 4**, the velocity was set to 5 mm/s (total time ~30 s).

A preliminary parametric study of TIA parameters was performed to determine the combinations for which the surface temperature does not exceed 45 °C. *E. coli* grow at temperatures between 7 and 50 °C (with optimal growth temperature of 37 °C), the temperature of 45 °C was chosen to avoid thermal damage to the bacteria. These measurements were conducted with temperature indicator strips (VWR temperature indicating strips 620-9102) and a temperature sensor (Pt100). **Figure 5** shows the position of these temperature sensors on the substrate in front of the plasma.

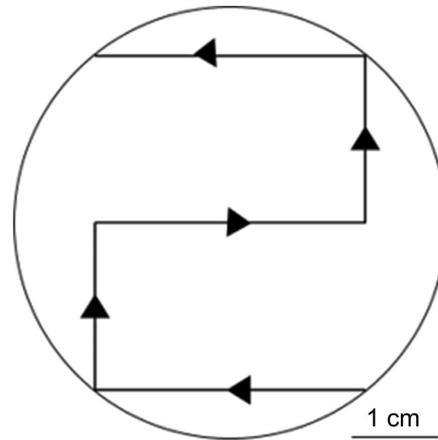


Figure 4. Diagram of the movement of the Petri dish in front of the plasma.

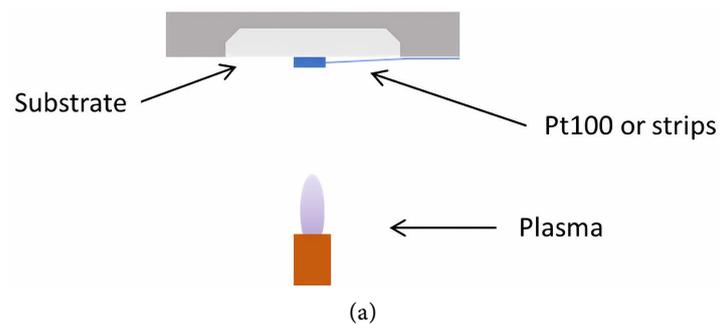


Figure 5. (a) Scheme of the position of the temperature sensors on the substrate; (b) temperature indicator strips after plasma exposure.

In addition, it was ruled out that there was no effect of the argon flow rate when projected on the inoculated Petri dish without producing plasma.

3. Results and Discussion

3.1. Influence of Process Parameters

The objective was to determine the influence of the process parameters on the decontamination efficiency. The results presented in this part were obtained without movement of the Petri dish.

The selected working conditions that do not exceed 45°C are listed in **Table 2**.

Table 2. Listing of the working conditions.

Flow rate (L/min)	Power (W)	Distance (mm)	Time (s)	Temperature (°C)*
3	200	35	10	45
5	200	35	10	37
5	200	35	20	37
5	200	35	30	37
7	200	30	10	43
7	200	30	20	43
7	200	30	30	43
7	200	35	10	37
7	200	35	20	40
7	200	35	30	40
10	200	30	10	40
10	200	30	20	40
10	200	30	30	43
10	200	35	10	37
10	200	35	20	37
10	200	35	30	37
7	250	35	10	40
7	250	35	20	40
10	250	35	10	37
10	250	35	20	40

*±1°C.

Surface temperatures lower than 45°C were obtained for a microwave power lower than 250 W, a nozzle-substrate distance of at least 30 mm and a maximum treatment duration of 30 s. The temperature of the substrate decreases with the increase in the flow of gas, the increase in the flow has the consequence of diluting the energy and of reducing the temperature of the plasma jet, consequently the thermal transfers towards the substrate. When a low power is injected, the substrate temperature is lower so to have a low temperature a low power and high gas flow rate are preferable. In addition, a high flow of argon could allow the introduction of air into the discharge by turbulence effect, which would increase the production of reactive species.

To evaluate the influence of the process parameters, the surfaces were measured after exposure to plasma and incubation (37°C, 24 h). As a bacterial lawn was formed on the agar, the colony count on the total surface was impossible, so the surface affected by the treatment was measured.

Figure 6 shows seeded Petri dishes, dish (a) is the control one and (b) has been exposed to the plasma. A uniform bacterial lawn was formed on the agar for the control sample exposed on the gas flow rate (**Figure 6(a)**). Gas flow rate therefore

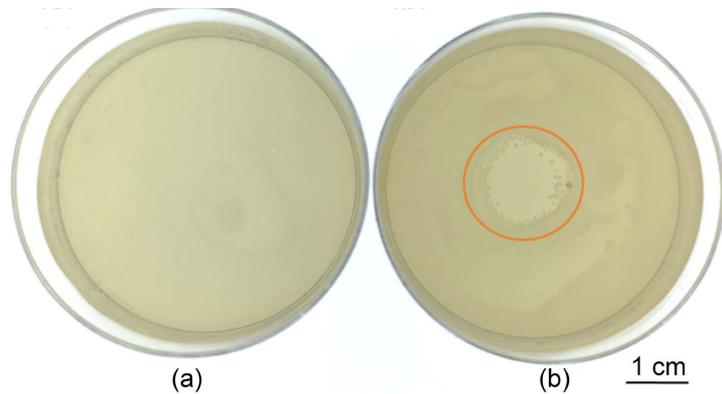


Figure 6. Pictures of Petri dish with 2.81.107 UFC deposited, (a) control, (b) plasma exposure (200 W, 7 L/min, 10 s, 35 mm) decontaminated surface 0.38 cm².

has no effect on bacterial growth. For the sample (**Figure 6(b)**), a bacterial lawn was formed except in the area affected by the plasma where there are fewer colonies. The decontaminated surface is identified by a circle, its surface was 0.38 cm².

Figure 7 shows the influence of the parameters on the measured decontaminated surface. Each condition was done twice, the average of the two was considered for CFU reduction counting.

An increase of the flow rate from 3 to 10 slpm and an increase of the microwave power from 200 W to 250 W gives an increase of the decontaminated surface from nearly zero to 1 cm² and from, 0.3 to 1 cm² respectively. These two parameters have a relatively low influence compared to the processing time. The decontaminated area was multiplied by 10 when the treatment time was increased from 10 to 30 s. It could be related to the increase of exposure to reactive oxygen and nitrogen species with the time. The influence of the distance was also evaluated (not presented here), the measured surfaces were similar for the two distances (30 and 35 mm) so the active species would still be present at 35 mm. But, the surface temperature was lower at 35 than at 30 mm. According to these results and in accordance with the literature, the most important parameter is the processing time [24].

Considering the findings reported up to this point, the measured temperatures and the plasma stability during movement, the operating conditions selected for the following measurements were: 200 W, 7 slpm and 35 mm ($T \approx 40^\circ\text{C}$). In this first part, the treatment was localized and thus evaluated by the measurement of the decontaminated surface. The setting in motion of the sample in the following part will make it possible to treat the entire surface of Petri dish, to count the number of surviving colonies after incubation and therefore to calculate a reduction rate.

3.2. Treatment Optimization: Substrate Movement

Petri dishes were exposed to the plasma under the conditions determined previously to calculate the reduction Log as a function of the number of passages of the plasma on the Petri dishes (**Figure 4**).

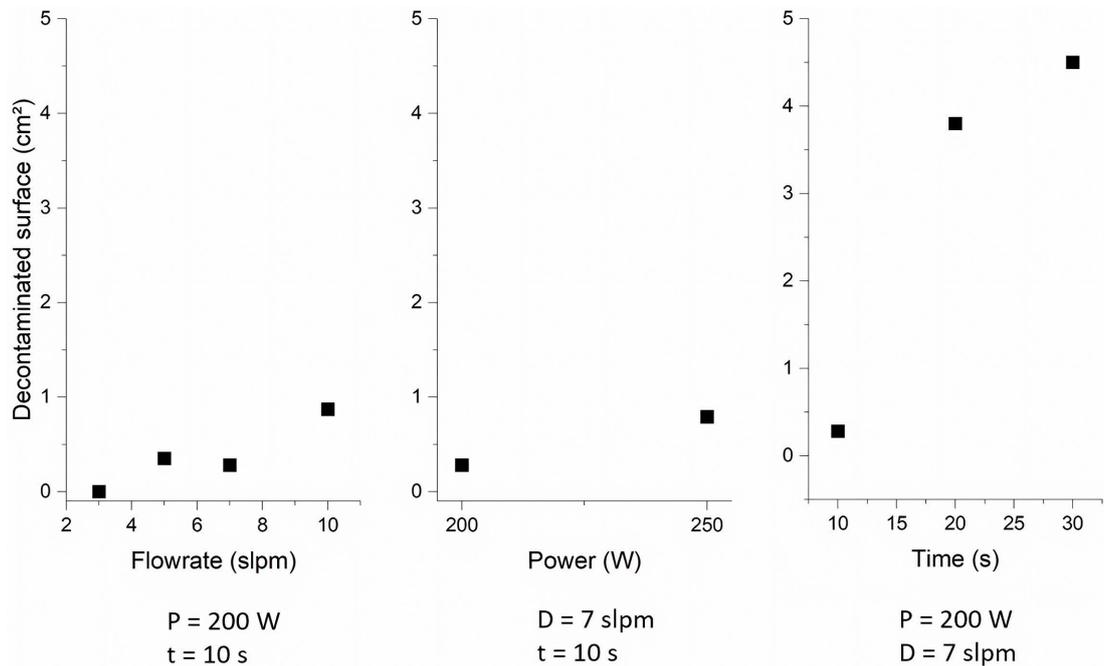


Figure 7. Effect of the process parameters on the decontaminated surface. Nozzle-substrate distance = 35 mm (the decontaminated surfaces are determined with an uncertainty of 0.05 cm²).

In order to evidence the role of UV, a quartz cover (QK16008, LabQua) was placed on the Petri dishes, so that the UV rays can reach the surface to be treated, but not the reactive species [24].

Figure 8 shows the *E. coli* reduction Log as a function of the number of passages in front of the plasma. All conditions were realised in triplicate to verify reproducibility. The reduction Log increased with the number of passages and was greater without the quartz cover, *i.e.* when the reactive species from the plasma were able to reach the surface. For the UV and species series, the reduction increased markedly while the increase is less marked between 2 and 3 passages for the UV series. 4.2 Log were obtained for the treatments with species and UV (3 passages), which corresponds to a disinfection process. The maximum temperature reached is 43°C, so the damage created to microorganisms is not due to a thermal effect.

The results in the literature vary depending on the gas and the surface (agar, glass, steel...) used for the deposition of bacteria, results are generally better on glass due to its low roughness. The reductions are ranging from 2 log to 7 log [24] [25]. For example, a reduction of 6 Log was obtained with a DBD on a glass pellet (10 mm diameter), this reduction is achieved in 13 minutes when the sample is placed at 1.5 cm and 40 minutes at 54 cm. Few studies have been carried out with a microwave source. However, Sato and al. used a coaxial microwave plasma flow, at 400 W and 5 L/min, a reduction of 2 log was obtained in 30 s [26]. The best results are obtained when the gas contains oxygen or nitrogen [27]. The TIA generates a voluminous plasma than other microwave plasma torches, allowing larger surfaces to be treated in a short time.

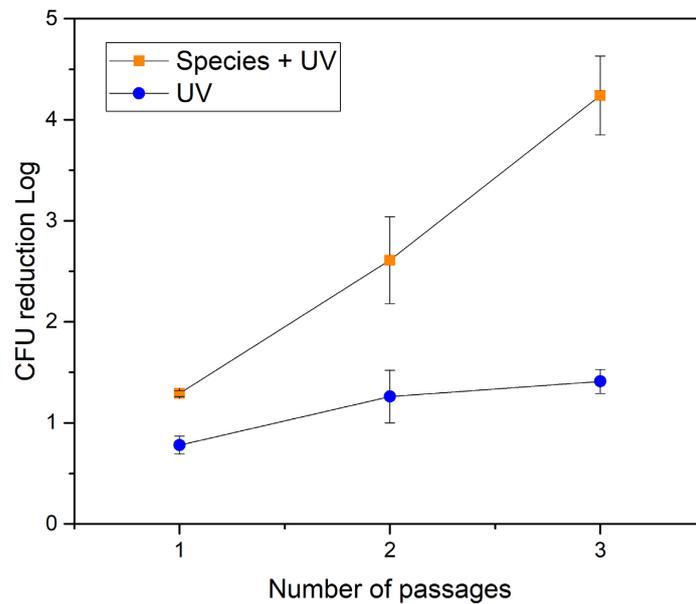


Figure 8. CFU reduction Log of bacteria as function of the number of passages, for UV and UV + species.

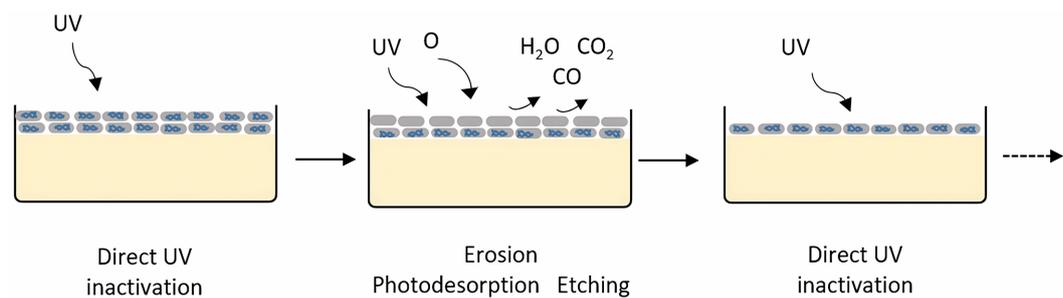


Figure 9. Mechanisms predominantly acting in each step from [26].

The small effect of UV could be explained by the low quantity of UV emitted by the plasma. But it can be also considered that the UV and active species produced in the plasma have complementary action in the damage caused to microorganisms. In fact, UV rays could cause the damages on the DNA of bacteria and the plasma active species play a role of erosion [5]. Erosion results from the adsorption of reactive species from the plasma on the bacteria with which they subsequently undergo chemical reactions to form volatile compounds (H_2O , CO_2 , CO ...). **Figure 9** shows the principal mechanisms to the inactivation of bacteria in a plasma environment [26].

In our case, the bacteria form several layers because of their concentration so the UV could inactivate the first layer, however without the plasma species the UV could not reach the second layer of bacteria. The plasma torch used in this study works at atmospheric pressure therefore the ambient air is introduced into the plasma at the nozzle outlet, due the turbulence in the periphery of the plasma. In previous work [28], species from oxygen and nitrogen (N_2 , N_2^+ , N^* , O^*) have been detected by optical emission spectroscopy (**Figure 10**). These active

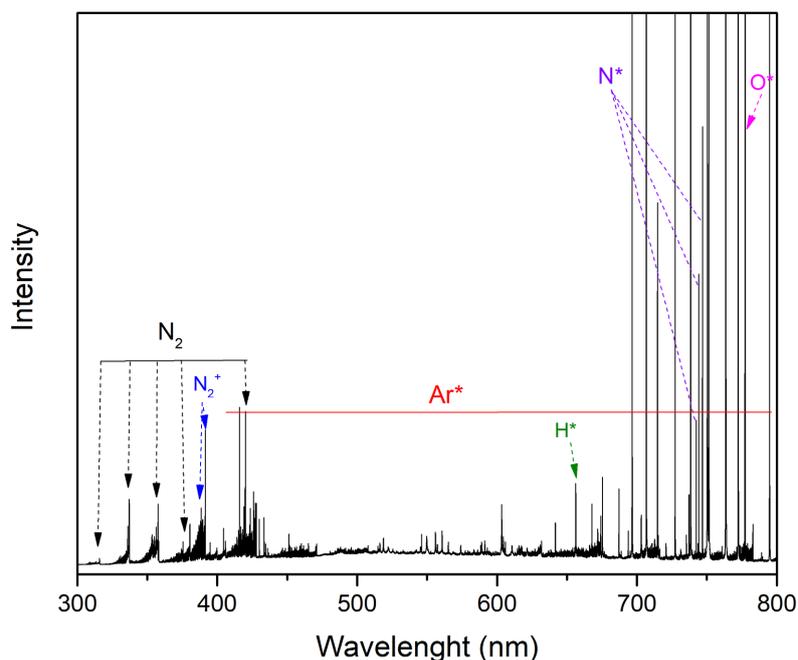


Figure 10. Global emission spectrum of argon plasma over the wavelength range 300 - 800 nm for 200 W/7 slpm (Spectra Pro 2750 Princeton Instruments).

species produced inside the plasma would allow the disinfection by complementing the UV role. Reactive oxygen and nitrogen species (ROS/RNS) can damage bacterial, plant, fungal and animal cell structures, this phenomenon is called oxidative stress.

4. Conclusion

The aim of this paper was to evaluate the ability of the TIA to inactivate the microorganisms without thermal effect. Among the working parameters—the process—distance, input power, gas flow rate and exposure time, the latter was the main factor influencing the reduction of *E. coli* cells. Preliminary study with static samples pointed out that the diameter of disinfection can be multiplied up to 10 with the time. Then, the moving of the samples during the treatment allowed to calculate CFU reduction Log. The primordial role of the reactive species of the plasma has been demonstrated and 4.2 Log reduction are obtained when the samples are submitted to the cumulating effects of reactive species and UV, 1.4 Log reduction are obtained with only UV. Therefore, the disinfection grade has been reached in the optimal conditions while maintaining a temperature of treatment lower than 45°C. TIA is a versatile process which also makes it possible to produce thin layers of TiO₂. The inactivation results obtained by plasma could be improved by coupling the effects of the plasma to those of the photocatalysis generated by TiO₂.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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