

## Inactivation of *Escherichia coli* in water by pulsed dielectric barrier discharge in coaxial reactor

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### ABSTRACT

An experimental study of ATCC (American Type Culture Collection) 8739 *Escherichia coli* bacteria inactivation in water by means of pulsed dielectric barrier discharge (PDBD) atmospheric pressure plasmas is presented. Plasma is generated by an adjustable power source capable of supplying high voltage 25 kV pulses,  $\sim 30 \mu\text{s}$  long and at a 500 Hz frequency. The process was conducted in a  $\sim 152 \text{ cm}^3$  cylindrical stainless steel coaxial reactor, endowed with a straight central electrode and a gas inlet. The bacterial concentration in water was varied from  $10^3$  up to  $10^8$  *E. coli* cells per millilitre. The inactivation was achieved without gas flow in the order of 82% at  $10^8$  colony-forming units per millilitre (CFU  $\text{mL}^{-1}$ ) concentrations in 600 s. In addition, oxygen was added to the gas supply in order to increase the ozone content in the process, raising the inactivation percentage to the order of 90% in the same treatment time. In order to reach a higher efficiency however, oxygen injection modulation is applied, leading to inactivation percentages above 99.99%. These results are similarly valid for lower bacterial concentrations.

**Key words** | atmospheric pressure plasmas, bacterial inactivation, ozone, pulsed dielectric barrier discharge, water

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### INTRODUCTION

The availability of fresh water, the most important chemical compound for human survival, is rapidly decreasing. In many emerging countries, improvements in water treatment have led to a near eradication of acute health hazards caused by waterborne diseases. Chlorination is the traditional water treatment disinfection method used around the world. It is a quite efficient disinfectant against many enteric bacteria. The use of chlorination has been decreasing, mainly due to toxic, mutagenic and/or carcinogenic disinfection byproducts (DBP) and chlorine residuals formed in the disinfection process (Koivunen & Heinonen-Tanski 2005). However, several wastewater treatment technologies for bacterial inactivation, which avoid the use of hazardous chemicals, are currently being applied (Fisher *et al.* 2008).

Ultraviolet (UV) irradiation is an important procedure for water disinfection. UV disinfection typically eliminates enteric bacteria effectively without producing DBP. The disadvantage of this method is its lack of bacteriostatic effect and the possibility for photoreactivation of UV-damaged microorganisms, enabling regrowth of the microbial population under certain conditions (Koivunen & Heinonen-Tanski 2005). Recently, several experiments have shown the effectiveness of applying dielectric barrier and pulsed plasma discharges to bacteria inactivation, both in fluid media and on general surfaces (Sato 2008; Ayan *et al.* 2009). The most distinctive characteristic of these discharges is their direct contact with the targets. It makes the treatment more effective, as long as the discharge is an abundant source of chemically active species wherever it

takes place in oxygen or air (Feng *et al.* 2007). The discharge generates UV light and other electromagnetic fields that facilitate collateral physico-chemical phenomena, which increases the synergy of the inactivation process.

These physical and chemical processes therefore act together to degrade or inactivate biological cells and chemical compounds, and the combination of electrical discharges with conventional disinfectants such as ozone and/or hydrogen peroxide might lead to a synergistic lethal effect. It has been shown that water treated by plasma contains a given amount of residual disinfectant capability, even when the plasma has been turned off, maintaining an inactivation ability that resembles a chlorine residual (Chen *et al.* 2009). However, carrying out these discharges can be complex when the material under treatment is a liquid because the electric field required for the plasma generation (in the range  $250 \text{ kV cm}^{-1}$  to more than  $10^3 \text{ kV cm}^{-1}$ ) widely exceeds that required for the same process out of gases (e.g. in air at room pressure, the field is  $\sim 30 \text{ kV cm}^{-1}$ ; Katsuki *et al.* 2002). Electric discharges in water are environmentally friendly and have proven to be more effective than conventional oxidants and disinfectants. However, the energy consumption of the electrical discharges generated in water is many times higher than that generated in gas (Chen *et al.* 2009) given that high-voltage pulsed discharges are required in order to attain breakdown conditions and sustain them for periods from nanoseconds to a few microseconds in order to avoid power losses, arcing and heating (Pokryvailo *et al.* 2004).

The pulsed dielectric barrier discharge (PDBD) technique combines, as expected, the main advantages of pulsed and of dielectric barrier discharges. Studies have been published on PDBD aspects such as: discharge techniques (Gao 2006); predictions based on numerical simulation (Albarello *et al.* 2008); generation and diagnostics equipment design (Rodríguez *et al.* 2008); as well as applications to areas such as medicine (Kalgatgi *et al.* 2007) and the environment (Mok *et al.* 2007).

The present report aims to show the degree of effectiveness of PDBD in the inactivation of ATCC (American Type Culture Collection) 8739 *Escherichia coli* (*E. coli*) bacteria in water. The equipment proposed for this experiment has been implemented around the high-voltage pulsed power supply of a cylindrical reactor endowed with a dielectric

between coaxial electrodes. Some authors have reported on the enhanced effect of electric discharges on bacterial inactivation when a constant gas flow is pumped into the liquid during the treatment (Zhang *et al.* 2006; Sato 2008; Chen *et al.* 2009). We propose the use of a modulated gas flow injected as the discharges are being conducted in samples of water. The discharges are then carried out without any gas flow. Finally, the treatment is performed adding a regulated continuous oxygen gas flow in order to compare the influence of either absence of gas, constant flow or modulated gas pulses on the effectiveness of the process.

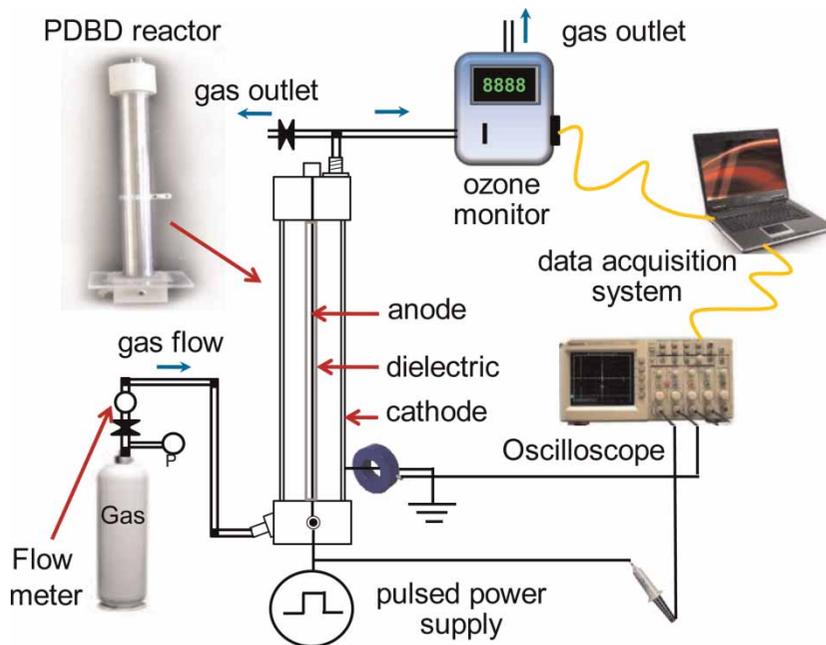
## METHODS

### Treatment chamber and electric system

The discharge reactor provides a  $\sim 152 \text{ cm}^3$  stainless steel encased cylindrical space whose symmetry axis is occupied by a tungsten rod covered with dielectric alumina; a gas inlet and a flow meter have been assembled at the lower end. Figure 1 is a schematic view of the experimental set-up including the adjustable 100–1,000 Hz power supply whose pulse width is 1–50  $\mu\text{s}$ , operating at 1–30 kV. The reactor configuration, main diagnostics and data acquisition systems are also displayed. An oscilloscope (TDS 2024, Tektronix, Inc.) with 200 MHz bandwidth, a 1:1,000 high voltage probe (Tektronix P6015A  $1000 \times 3.0 \text{ pF } 100 \text{ M}\Omega$ ) and a current transformer (Stangenes 2-0.1W) were used to register the voltage and current waveforms.

### Bacteria preparation

The *E. coli* populations required by the study were developed as samples of ATCC 8739 strain inoculated in 5 mL of Luria-Bertani (LB) liquid broth and incubated for 24 h at  $37^\circ\text{C}$ , being continuously stirred (overnight culture). The culture was centrifuged for 10 min at 5,000 rpm, twice. The sedimented bacteria were put in a 5 mL suspension of distilled and sterilized water. In order to determine the initial concentration of *E. coli* cells obtained during the overnight culture, an aliquot of 0.1 mL at  $1:10^2$  dilution from the original aliquot was made and the cells were counted in a Neubauer chamber (Neubauer improved)



**Figure 1** | Experimental layout of the PDBD treatment of *E. coli* in culture water.

with a depth of 0.100 mm and a 0.04 mm<sup>2</sup> area per square by means of a phase contrast microscope. The concentration in cells per millilitre (cells mL<sup>-1</sup>) was determined by:

$$\text{cells mL}^{-1} = \frac{\text{counted cells} \times 1,000}{\text{square area} \times \text{chamber depth}}$$

where the counted cells numerator is the average of total cells counted per square and 1,000 is the factor to convert mm<sup>-3</sup> into mL<sup>-1</sup>. The cells counted by this method are ~10<sup>7</sup> cells mL<sup>-1</sup> and the original concentration can be determined by multiplying it by the dilution factor, leading to ~10<sup>9</sup> *E. coli* cells mL<sup>-1</sup>. The final concentration for each experiment was obtained following this method and adjusted by progressive dilutions.

### Experimental conditions

The power supply output required by the discharges conducted in water was established at 25 kV and 500 Hz, with a ~30 μs pulse width. In each experiment, 15 mL distilled water samples containing controlled concentrations of 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>6</sup> and 10<sup>8</sup> *E. coli* cells mL<sup>-1</sup> were placed into the reactor. Ten experiments were carried out with each

concentration while an untreated sample is kept as a reference. The reactor gas inlet was kept closed during experiments without gas flow. In order to improve the production of reactive oxygen species (ROS), two cases were considered. In the first, oxygen gas was added at a 0.5 L min<sup>-1</sup> flow rate in a continuous mode during each treatment period. In the second, an intermittent 0.5 L min<sup>-1</sup> flow of oxygen was admitted for 15 s periods every 30 s until the end of the treatment period. The 0.5 L min<sup>-1</sup> oxygen gas intake flow optimized the production of ozone under the experimental conditions. Data acquisition of ozone concentration values along the process was conducted by sampling at 0.5 Hz throughout every experiment. Immediately after plasma treatment, the samples were transferred to sterile tubes after which the microbiological analysis was carried out.

### Microbiological analysis

Three Petri dish (90 mm diameter) sets per sample at 10<sup>5</sup> *E. coli* cells mL<sup>-1</sup> concentrations (undiluted) in LB agar were compounded by inoculating 0.1 mL in each dish using a spread-plate technique (Gupta 2007). Samples were taken from both untreated (initial concentration of *E. coli*)

and treated samples. Once labelled, the samples were incubated at 37 °C for 24 h periods. For  $10^4$ ,  $10^6$  and  $10^8$  *E. coli* cells  $\text{mL}^{-1}$  concentrations, samples of  $10^3$  cells  $\text{mL}^{-1}$  were obtained from a standard dilution assay from the untreated sample. In order to determine its initial concentration, 0.1 mL of the dilution was spread onto Petri dishes containing agar medium. Inoculated dishes were incubated overnight at 37 °C for 24 h periods. For  $10^4$ ,  $10^6$  and  $10^8$  *E. coli* cells  $\text{mL}^{-1}$  concentration treated samples, dilutions from 1:10 to 1:10<sup>5</sup> were spread in triplicate directly onto the surface of LB agar and the cultivation method described below was applied.

After overnight incubation, the number of *E. coli* colonies was estimated as the number of surviving cells, assuming that every viable bacteria results in the formation of a colony (Lee 2009). Colony count was performed for the entire area of each plate sample and concentrations of colony-forming units (CFU) were calculated. This result was then multiplied by 10 in order to obtain the number of CFU per millilitre (CFU  $\text{mL}^{-1}$ ). The survivability percentage was calculated as follows (Zhang *et al.* 2006):

$$\text{Survivability \%} = \frac{N_t}{N_0} \times 100\%$$

where  $N_0$  is the initial *E. coli* concentration in CFU  $\text{mL}^{-1}$  and  $N_t$  is the *E. coli* concentration after plasma treatment in CFU  $\text{mL}^{-1}$ . These results are presented either as the mean value or the mean and standard deviation of ten experiments.

### Chemical diagnostic

In order to identify the chemical species present in the discharge, optical emission spectroscopy (OES) was performed over the 270–870 nm wavelength range using a Jaz OceanOptics™ spectrometer with a maximum optical resolution of ~0.3 nm (full width at half-maximum or FWHM).

The concentration of ozone was monitored during each experiment by means of a UV absorption ozone meter (Ozone Monitor Model 460 L Teledyne Instruments™) operating at 253.7 nm. Data acquisition from the ozone monitor was processed on LabView™ software and plotted against treatment time.

## RESULTS AND DISCUSSION

### Discharge and chemical species

The PDBD voltage and current waveforms are depicted in Figure 2. The latter presents peaks indicating streamer formation in the reactor, which is limited by the dielectric therefore avoiding arcing and maintaining the typical behaviour of a pulsed discharge (Rodríguez *et al.* 2008). Energy consumption per pulse (mJ pulse<sup>-1</sup>) was calculated by numerical integration of the voltage and current waveforms resulting in 100 mJ pulse<sup>-1</sup>. These electric discharges produce ozone and ROS. In particular, breaking down in water generates powerful oxidation species such as the hydroxyl radical OH (Feng *et al.* 2005; Kornev *et al.* 2006; Ono & Oda 2008).

Results of OES measurements are shown in Figure 3, which displays the emission bands from the discharge with and without oxygen gas flow. Figure 3(a) presents the respective emission spectra: mainly from hydroxyl radical (OH) in the 306.3 nm band ( $A^2\Sigma^+ \rightarrow X^2\Pi$ , 0-0), the band of the N<sub>2</sub> second positive system (337.1 nm,  $C^3\Pi_u \rightarrow B^3\Pi_g$ , 0-0), the N<sub>2</sub><sup>+</sup> first negative system (358.2,  $B^2\Sigma_u^+ \rightarrow X^2\Sigma_g^+$ , 1-0), the N<sub>2</sub> second positive system (380.4 nm,  $C^3\Pi_u \rightarrow B^3\Pi_g$ , 0-2), H<sub>α</sub> (656.3 nm,  $3d^2D \rightarrow 2p^2P^o$ ), and O (777.4 nm,  $3p^5P \rightarrow 3s^5S^o$ ) produced by the PDBD in H<sub>2</sub>O without oxygen flow. It should be observed that the spectra are dominated by N<sub>2</sub> transitions.

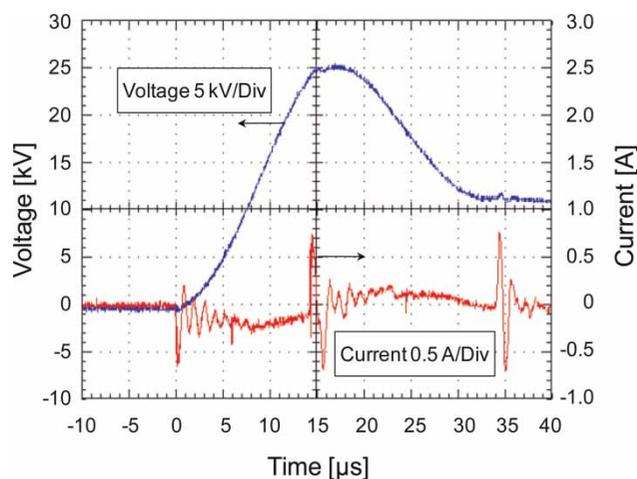
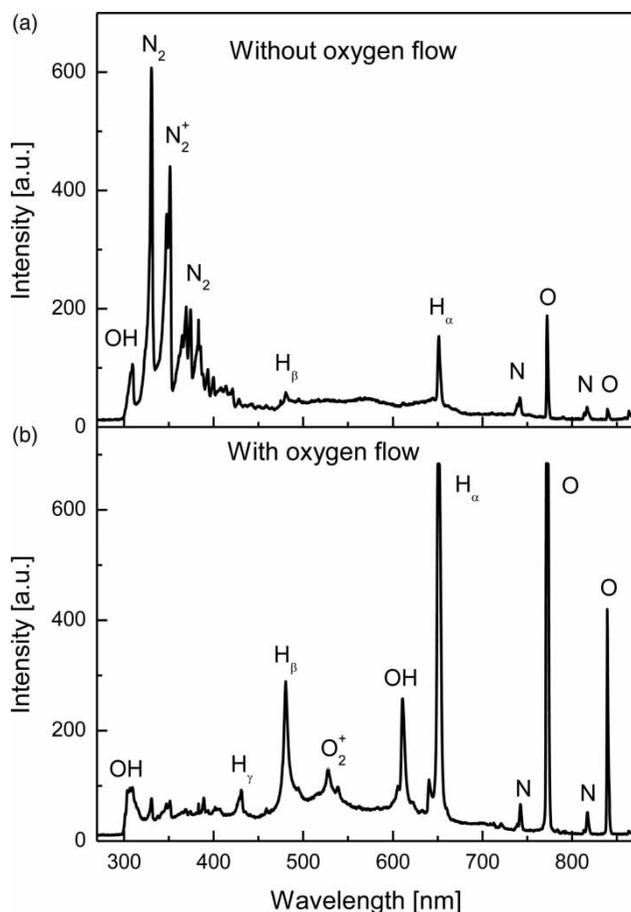


Figure 2 | Typical voltage and current waveforms of a PDBD in water.



**Figure 3** | Typical emission spectrum in PDBD in water (a) without oxygen gas flow and (b) with oxygen gas flow into the reactor.

The effects of the addition of  $O_2$  on the emission intensities of  $H_\gamma$  (434 nm,  $5d^2D \rightarrow 2p^2P^o$ ),  $H_\beta$  (486.1,  $4d^2D \rightarrow 2p^2P^o$ ),  $H_\alpha$  (656.3 nm,  $3d^2D \rightarrow 2p^2P^o$ ), O (777.4 nm,  $3p^5P \rightarrow 3s^5S^o$ ) and O (844.6 nm,  $3p^3P \rightarrow 3s^3S^o$ ) are evident. **Figure 3(b)** shows the strong emission observed in the  $O_2^+$  first negative system ( $529.5, b^4\Sigma_g^- \rightarrow a^4\Pi_u, 2-0$ ) and the OH radical in the band of 613.7 nm ( $X^2\Pi, 5-0$ ), exhibiting a significant difference from **Figure 3(a)**.

In order to understand the difference between spectra, we will discuss the production processes of O, H and OH radicals under the condition of added oxygen. The additional  $O_2$  can be dissociated through reactions (1)–(3) in a PDBD, while considerable proportions of O ( $^1D$ ) and O atoms can be produced by the following pathways (Feng *et al.* 2005):



The main pathway leading to OH radical formation by discharge is thought to be the result of the direct dissociation of water molecules by electron impact, leading the generation of H and O:



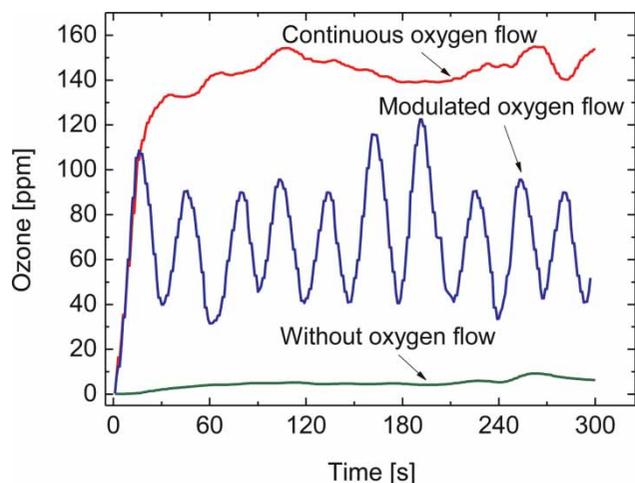
However, it has also been reported that the generation of OH radicals increases along with the concentration of  $O_2$  molecules (Ono & Oda 2008). Likewise, a lot of OH radicals can be produced by the interaction of  $O(^1D)$  and  $H_2O$ :



The primary species produced by an electrical discharge occurring in either air or oxygen is ozone. It is the only active species that can diffuse into the liquid phase and react with aqueous contaminants that are susceptible to degradation by direct attack (Lukes *et al.* 2004). Ozone can therefore react in an aqueous medium both directly, e.g. as molecular ozone, or indirectly via radical-intermediates formed during ozone decomposition in the aqueous media. Hydroxyl radical is the most important species formed during ozone decomposition.



Curves of ozone concentration in ppm were obtained from all experiments and are depicted in **Figure 4**. Concentration was saturated between 140 and 155 ppm in continuous oxygen flow while, with modulated gas,



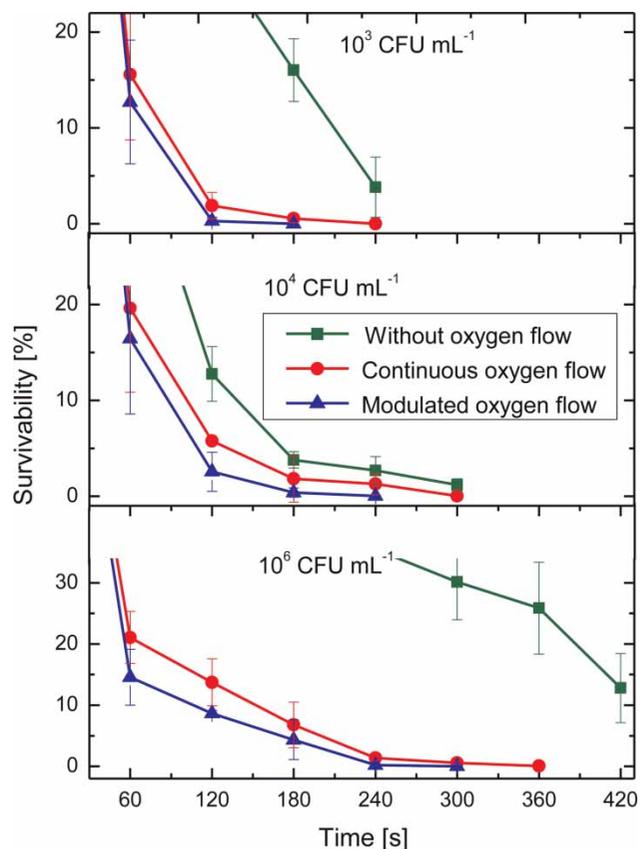
**Figure 4** | Curves of ozone concentration without oxygen flow and with continuous and modulated oxygen flow.

saturation did not exist. The ozone level measured in the process without gas flow is <10 ppm.

### *E. coli* inactivation

The kinetics of bacterial inactivation was estimated from the survivability percentage of *E. coli* bacteria versus the total plasma treatment time. Figure 5 exhibits the main results of the mean and standard deviation of the inactivation process at  $10^3$ ,  $10^4$ ,  $10^6$  CFU mL<sup>-1</sup>, without gas and with either continuous or modulated gas flow.

The survival curves in Figure 5 indicate that gas modulation increased the efficacy of the process. In particular, 99.99% inactivation was achieved at a  $10^3$  CFU mL<sup>-1</sup> concentration after 180 s of modulated oxygen gas flow PDBD. The same result was obtained with oxygen gas flow in continuous mode after 240 s, but only 96% inactivation was achieved without gas flow in the same period. With  $10^4$  CFU mL<sup>-1</sup> of bacteria, 99.89% inactivation was achieved at 240 s in modulated gas flow, approximately the same order was obtained after 300 s in continuous gas flow and 98% in 300 s without gas flow in the reactor. In samples at  $10^6$  CFU mL<sup>-1</sup> concentrations, 99.98% inactivation was obtained at 300 s with modulated oxygen flow whereas 99.96% of inactivation was obtained at 360 s with continuous flow; 87% inactivation was however achieved after 420 s without gas flow.

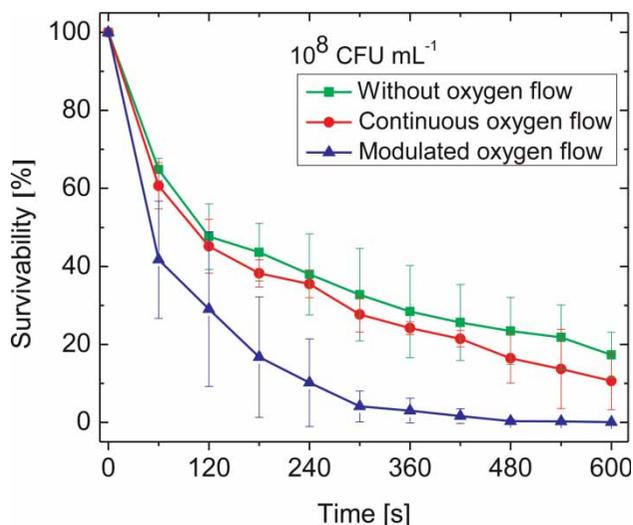


**Figure 5** | Comparison of *E. coli* survival curves with respect to oxygen gas flow mode.

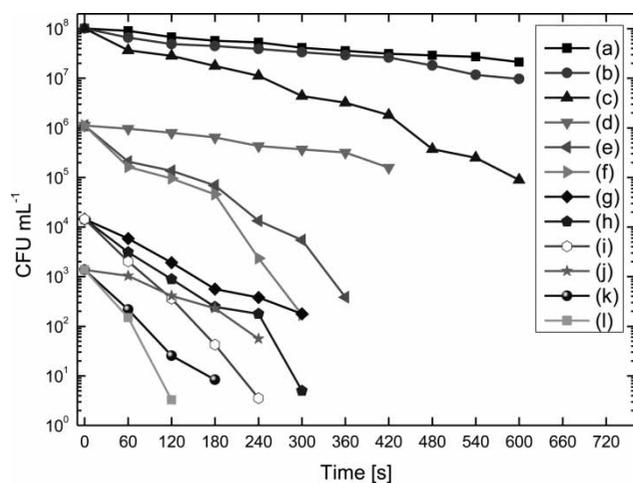
In all cases, curves with modulated gas flow achieved higher inactivation efficiency than the continuous flow or the absence of gas. A similar efficiency with  $10^3$  CFU mL<sup>-1</sup> was achieved in 25% less time with modulated oxygen flow and approximately the same efficiency was achieved in 20% less time with a modulated flow at  $10^4$  and  $10^6$  CFU mL<sup>-1</sup> concentrations.

The outcome of treating  $10^8$  CFU mL<sup>-1</sup> concentration samples for periods up to 600 s is displayed in Figure 6. It is again remarkable the difference in efficacy of the process when conducted with and without gas flow, and with continuous or modulated flow. The effectiveness of the resulting elimination process of *E. coli* at high bacterial concentrations, as seen in Figure 6, reaches 82% without gas flow, ~90% with constant gas flow and, in a gas modulation regime, up to 99.99%. This technique is similarly viable at smaller bacterial concentrations.

Figure 7 presents the way in which the use of modulated gas flow injection raises the lethality in all cases. Thus, a



**Figure 6** | *E. coli* survival curves at  $10^8$  CFU mL<sup>-1</sup> in PDBD process without oxygen flow and continuous and modulated oxygen flow.



**Figure 7** | Evolution of *E. coli* inactivation by PDBD treatment with the following initial conditions: (a)  $10^8$ , (d)  $10^6$ , (g)  $10^4$  and (j)  $10^3$  CFU mL<sup>-1</sup> without gas flow; (b)  $10^8$ , (e)  $10^6$ , (h)  $10^4$  and (k)  $10^3$  CFU mL<sup>-1</sup> in continuous oxygen flow; and (c)  $10^8$ , (f)  $10^6$ , (i)  $10^4$  and (l)  $10^3$  CFU mL<sup>-1</sup> in modulated flow.

reduction  $>2$  log is attained at concentrations of  $\sim 10^3$  CFU mL<sup>-1</sup> and, although similar reductions are achieved at  $\sim 10^4$  CFU mL<sup>-1</sup>, these are extended up to  $>3$  log when the treatment period is extended to 300 s. At  $\sim 10^6$  CFU mL<sup>-1</sup> the reduction is  $>3$  log with 360 s or  $\sim 4$  log when the oxygen gas is modulated for 300 s. Finally, if concentrations as high as  $10^8$  CFU mL<sup>-1</sup> are chosen,  $>2$  log reductions demand 600 s periods with modulation, otherwise the times extend much longer.

These findings can be interpreted as active chemical species being formed during the gas-on events, which react with the environment as soon as the flow is suppressed and where one or more active species interact with the cytoplasmic membrane of *E. coli*, consequently suppressing the bacterial activity. Similar observations have been reported by Sato (2008) and Chen et al. (2009). In these cases, the authors interpreted that active species could be blown off once the gas flow rate was high enough. Likewise, ROS and OH radicals are suggested to play an important role in the bacterial inactivation and to cause physical destruction of the cytoplasmic membrane by oxidation after plasma exposure (Pompl et al. 2009). According to this hypothesis, a saturation of O atoms and O<sub>3</sub> molecules produced by the added O<sub>2</sub> can react with OH radicals as follows (Feng et al. 2007):



Although a vast number of OH radicals are formed with H and HO<sub>2</sub> reacting with O<sub>3</sub>, a saturation of O and O<sub>3</sub> therefore counterbalances this effect.

The effectiveness of the PDBD method can be compared to disinfection treatment with ozone where a 99–99.99% of *E. coli* inactivation was achieved under certain conditions (Hunt & Mariñas 1999). The advantage of the modulated flow method is that ozone is generated inherently to the discharge. The PDBD seems to be more energy efficient compared to another electrohydraulic discharge system, where pulsed corona discharges employ energy of the order 1 J pulse<sup>-1</sup> while the pulsed arc discharge needs energy of the order 1 kJ pulse<sup>-1</sup> and larger (Chang 2009). Although the high price of pure oxygen increases the cost of the PDBD treatment, oxygen could be substituted with air in order to increase the competitiveness and therefore make bacterial inactivation in water methods based on PDBD economically feasible. Further research is required to advance the understanding and optimize the operating cost of this technology.

## CONCLUSIONS

A PDBD technique in water has been executed successfully, proving that its application is efficient for bacterial inactivation even at high concentrations and, consequently, relevant for water purification. The optical emission spectra of chemical species in the discharges in water, with and without oxygen flow, have been observed successfully. The possible reason for a large number of N<sub>2</sub> molecules excited in the plasma, as shown in Figure 3(a), is the presence of air in the reactor. When oxygen is added the air is displaced, emitting weak spectra in the N<sub>2</sub> wavelength region from 337.1 to 380.4 nm. It was observed that the dominant emission region of the OH radical is around 613.7 nm when oxygen is added; a relationship between the presence of the OH radical and the bacterial inactivation efficiency is suggested. The enhanced effectiveness of the modulated oxygen intake discharges can be attributed to the prevention of gas saturation as well as to an improved ROS–water reaction leading to the production of more free radicals through different chemical pathways. By contrast, saturated oxygen and ozone may react with the free OH radicals, which counterbalance the bactericide activity. This finding is highly relevant for future applications of electrical discharges in bacterial inactivation in water.

## ACKNOWLEDGEMENTS

This work benefited from financial support from CONACYT, Mexico. The authors are obliged to: I. A. Rojas O., M. T. Torres M., P. Angeles E., I. Contreras V. and M. Pacheco P. for their technical support.

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First received 15 August 2011; accepted in revised form 10 April 2012. Available online 30 May 2012