Microbiological treatment of water by cold plasma at atmospheric pressure

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Abstract
Ensuring the world's population access to drinking water is one of the millennium's challenges, for although our planet is made up of 70% of water, poor distribution and water quality are problems that concern world leaders, being among the objectives of development 2030 agenda set by the ONU. The proposed work aimed at the microbiological treatment of water through cold plasma technology at atmospheric pressure, the control to confirm the efficiency of the treatment was through the analysis of thermotolerant coliforms. The reduction was 93.33% for thermotolerant coliforms and cold plasma technology reduces the time of microbiological treatment of water by 90% over traditional methods due to high energy density, ozone generation and ultraviolet radiation, thus reducing costs because the energy expenditure was low and there is no need to add chemicals.
Keywords: microbiological treatment; cold plasma; non-thermal plasma; *E. coli* inactivation; air plasma gas

1. Introduction

New methods and processes able to remove chemical and microbiological contaminants from water becomes more and more important and several authors have already demonstrated that non-thermal plasma technology is very efficient (Bruggeman et al. 2016, Li et al., 2015).
Non-thermal plasma applied to bacteria inactivation in water is considered economical and safe, also it avoids the addition of chemicals such as chlorine that can react with organic matter in water medium and produce carcinogenic compounds. In addition, the inactivation process of the microorganisms by non-thermal plasma also prevents multidrug-resistant organisms.

Atmospheric pressure plasma technology was firstly used for the sterilization process. Recently, studies have shown excellent results of Escherichia coli (E. coli) inactivation in contaminated water by both discharge plasma on the aqueous surface and by plasma jet immersed in the liquid medium (Ke et al., 2017, Mortazavi et al., 2016). Although the mechanism of action of plasma in different microorganisms' inactivation, is still not well understood, it has been successfully applied (Leclaire et al., 2008) and its efficacy differs due to differences in membrane structure (Lu et al., 2014)
Therefore, the main objective of this work was to study the inactivation of E. coli in water by non-thermal plasma reactors using air and Ar as plasma gas.

2. Experimental Conditions

The experiments were carried using a non-thermal plasma reactor (figure 1) AC high voltage source (± 17 kV) and 30 mA current for output maximal power of 510 W and plasma gas flow rate of 3.0 L min^{-1}. The reactor working at atmospheric pressure with 60 mL of distilled water pre-inoculated with E. coli bacteria kept under electromagnetic stirring. The treatment time used was 10 min and samples of 100 µL were collected every two min and all experiments were performed in triplicate. pH, conductivity and temperature were checked and controlled every two minutes during experiments using HANNA Instruments HI 9828 multi parameter equipment. The plasma reactors were cleaned and sterilized with ethanol 70% and dry in a laminar flow hood under UV radiation for two hours. The bacteria colonies used were counted in UFC mL^{-1} (Colony Forming Unit).

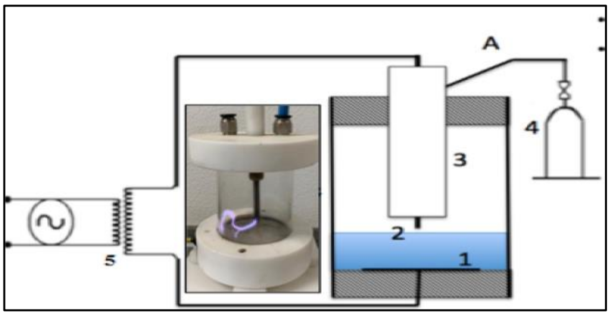

Figure 1. Schematic drawing of the RFG and RFL type plasma reactors: 1 - Aluminum electrode; 2 - Tungsten electrode; 3- Electrodes support; 4 – Gas supply, compressed air or Ar; 5 - power supply

3. Results

Table 1 show the results obtained over 10 min, both in the control test (time zero) and in the tests performed under non-thermal plasma treatment using air and Ar as plasma gas.
The results of the inactivation of the E. coli colonies presented in table 1 show ~100% of bacteria inactivation in 6 min of plasma treatment operating with air as a plasma gas. For the Ar as the plasma gas the results show that after 10 min 48 CFU ml^{-1} still remaining active. This result indicates that chemicals species derived from air plasma are much more effective in bacteria inactivation than species derived from Ar plasma.

The results of pH, conductivity and temperature Table 1 show that using air as the plasma gas the pH of the medium decreased ~3.7 units with the treatment time of 10 min and the conductivity increase ~750 units from 100 to 850 µScm^{-1}. The air plasma produces in water nitrite species which lowered pH of the medium and the combination of the ionic species formed increase the conductivity. The temperature of the medium increased by 10 ^{o}C in 10 min which is related to the energy transferred from the ionized gas to the water.

Table 1 shows the result using Ar as the plasma gas in the reactor, there was practically no variation in pH and conductivity, and the temperature variation was lower than that observed for the same RFG reactor using air as the plasma gas. Species generated by the Ar plasma show that the formation of active species in water and their effect on bacteria inactivation are less important than the direct formation of species derived from nitrogen and oxygen in water (equations 4 to 16). The temperature variation of the medium was also lower with Ar of only 6 ^{o}C, against 10 ^{o}C in 10 min for air, which shows that the species produced with Ar transfer less energy to the liquid medium than the chemicals species produced by air plasma.

Table 1. Results of pH, conductivity, temperature and bacteria inactivation by non-thermal plasma RFG-type reactor using air and Ar as plasma gas

Time (min)	**0**		**2**		**4**		**6**		**8**		**10**	
Gas	Air	Ar	Air	Ar	Air	Ar	Air	Ar	Air	Ar	Air	Ar
pH	6.65	6.71	4.74	6.87	3.78	6.73	3.12	6.59	3.02	6.48	2.91	6.35
Cond. (µS/cm)	100	154	182	153	274	166	633	182	715	194	853	187
Temp. (^{o}C)	26	26	32	30	34	31	35	32	36	32	36	32
CFU mL^{-1}	10^5	10^5	5.20 10^2	9.00 10^2	2.00 10^2	7.20 10^2	10^1	3.50 10^2	10^0	1.60 10^2	10^0	4.8 10^1
Images of CFU/ *E. coli.*												

4. Conclusions

The results showed that when air was used as non-thermal plasma gas showed better results than Ar, mainly in relation to the decrease in the count of bacteria E. coli. The air reactive species that diffuses in the liquid medium resulting in bacteria inactivation. When air was used as plasma gas, the solution pH decreased and conductivity increased indicating that the nitrates species that lowering the pH are the key species inducing bacteria inactivation in water. Bacterial inactivation is favored in acidic medium due to bacterial cells sensitive to the acid that affects intracellular components such as cells enzymes and proteins. Also nitrous acid react strongly with the plasma membrane and changes the selective permeability by influencing the proton motive force, which occurs due to the rapid influx of H^+ with the consequent increase in energy required to maintain its internal alkaline pH. The E. coli inactivation method using air non-thermal plasma showed to be very efficient, easy to operate and environmental friendly as well as non-thermal plasma prevents multidrug-resistant organisms as ordinary antibiotic may induces.

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