PDBD with continuous liquids flows in a discharge reactor

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Abstract. This paper presents the design, construction and testing of a cylindrical pulsed dielectric barrier discharge (PDBD) reactor aimed to microbiological elimination of *Escherichia coli* ATCC 8739 bacteria. In the reactor, water flowed continuously and to countercurrent an oxygen gas was injected. The water pumping was carried out with a peristaltic pump type, stainless steel and aluminum constructed, and water was recirculated through norprene tubing. The considered parameters in order to promote energetic efficiency were: the residence time of the water contaminated with bacteria, flow rate of the liquid, shape and material used to build electrodes and dielectric, pressure, and gas injection flow rate. The pulsed power supply parameters are featured by 25–30 kV high voltage, 500 Hz frequency and 30 μs width. The outcome elimination of *E. coli* bacteria at $10^3$, $10^4$ and $10^6$ CFU/mL concentrations reached an efficiency over 0.5 log-order in absence of oxygen; while >2 log-orders when oxygen gas was injected during the process.

Introduction

Water is an indispensable resource for living organisms. In 2002, United Nations Organization declared human right to water as fundamental, it implies have access to water in enough quantity and acceptable quality [1]. Nevertheless, according to the World Health Organization (2013), at global level, approximately 1.1 billion of people do not have access to water sources with an acceptable quality level; 2.4 billion of people do not have some sort of sanitation facility and close to 2 million of people, most of them children under five years old, die every year due to diarrheal disease [2]. In general, the main source of risk is associated with polluted water ingestion, which contains pathogen microorganisms from people and animals feces; therefore, the implementation of an appropriate disinfection process is essential.

Currently, the disinfection process widely implemented in Mexico corresponds to chlorination, in which have been registered responsible compounds to cause cancer such as organochloride compounds and trihalomethanes [3, 4]. Therefore, there is an urgent need to propose, develop and deploy efficient and safe processes as alternatives to chlorination for the elimination of pathogen microorganisms in water.
In recent years a particular interest has grown in the application of pulsed dielectric barrier discharges (PDBD) in water with the aim of achieving synergistic effects (production of reactive chemical species, pulsed electric fields, UV radiation and shock waves), in reduced conditions of time and under the implementation of a safe procedure for the microorganisms elimination in water. Currently, research works have been reported [5-12]; nevertheless, these investigations have been carried out maintaining a certain volume of liquid in a state of rest.

This work aims to evaluate the elimination efficiency of *Escherichia coli* (*E. coli*) bacteria ATCC 8739, which is widely used as indicator microorganism of fecal contamination in water, through a designed pulsed dielectric barrier discharge reactor coupled to a hydraulic system with a total treatment capacity of 500 mL, operating in recirculation mode under steady and laminar flow conditions at 293.15 K and 103 kPa.

2. Experimental setup and microbiological procedure

The experimental setup that was employed in antimicrobial treatment experiments is presented in figure 1. The following subsections are below described in detail:

**2.1 Description of Hydraulic Setup**

An experimental hydraulic setup was designed in order to operate in water recirculation, with a total capacity of 500 mL volume for the microbiological elimination of *E. coli* bacteria (see figure 1). The operating conditions were established under steady and laminar flow at 293.15 K of temperature and 103 kPa of pressure.

The experiment was started putting inside a vessel 500 mL of distilled water inoculated at $10^3$, $10^4$ and $10^6$ *E. coli* cells mL$^{-1}$ concentrations. Once started the application of discharges, a peristaltic pump type operating at 1.2 mL s$^{-1}$ was used for the liquid movement from the vessel with water inoculated with *E. coli* bacteria at the top of the reactor, where through an adapted piece, the liquid fluid was distributed inside the chamber to return to the first vessel and receive the treatment again.
2.2 Description of PDBD Reactor

The PDBD reactor is biased by a pulsed power supply, which is featured by pulse height 1-30 kV, frequency 100-2000 Hz and pulse width 0-30 µs; these parameters can be adjusted by the user. The pulse voltage and current signals from the PDBD reactor were picked up by a high voltage probe (Tektronix P6015A, 1000× 3.0 pF and 100 MΩ) and a Rogowski coil (Stangenes 2-0.1 W) (see figure 1). The signals were monitored with a digital oscilloscope (Tektronix TDS2024, 200 MHz-2 GS/s).

The cylindrical geometry PDBD reactor with a volume 38.0 mL constructed of stainless steel 316L (see figure 1) in order to avoid corrosion problems due to the production of reactive oxygen species during the discharge process; it also was used as cathode (see figure 1, point 1). According to figure 1, at the top of reactor vessel was inserted a nylon piece with a hole, which provides the inlet of liquid, which comes from the vessel with water inoculated with E. coli bacteria (point 2), distribute the liquid flow inside the reactor vessel (point 3); enable the outlet of gaseous chemical species generated during the elimination of E. coli bacteria (point 4) and, with an adaptation attached at the bottom (point 5), give an adequate and rigid support to dielectric and anode of the system (point 6). The selected material in order to construct the anode corresponded to a tungsten wire of 300 mm length, it was inserted coaxially to the reactor vessel and covered with an alumina hollow rod of 2.44 mm diameter and 350 mm length (point 5), the latter being the dielectric barrier (point 7). The outlet of liquid in treatment (point 8) was carried out by the lateral and inferior side of the reactor vessel. The treated liquid is received in the vessel that contains water inoculated with E. coli bacteria; here it is taken the sample to be analyzed microbiologically.

Due to temperature could influence in the elimination process of microorganisms, it is necessary an additional system to control it. Therefore, to maintain a constant temperature during the experiments, in the outside of the PDBD reactor vessel was coupled a cooling system (point 9), which consisted of an isolated jacket with water as working fluid, to avoid bacterial contamination was manufactured with PVC of diameter 25.4 mm and operated at recirculation mode. Additionally, the reactor vessel had a lateral adaptation (10), whose function was allow the oxygen gas to flow at 8.3 mL s⁻¹ inside it at countercurrent with the liquid flow.

2.3 Description of Microbiological Procedure

2.3.1 Bacterial growth and preparation. As before mentioned, the effectiveness bacterial elimination in water was determined using the strain of E. coli ATCC 8739 from the American Type Culture Collection bacteria, as the selected indicator microorganism to determine the process efficiency. For this, a strain was inoculated in 5 mL of Luria-Bertani (LB) broth and incubated for 86,400 s at 310.15 K, being continuously stirred (overnight culture). The cells were harvested by centrifugation (twice for 600 s at 5000 rpm) and then it adds 5 mL of distilled and sterile water. In order to determine the obtained initial concentration of cells after overnight culture, an aliquot of 100 μL at 1:10² dilution is considered, and the count of cells was performed by a phase contrast microscope and a Neubauer chamber. The concentration in cells per milliliter (cells mL⁻¹) was recorded and cells density at stationary phase was approximately 10⁷ cells mL⁻¹. The original concentration can also be approached multiplying it by the dilution factor, leading to ~10⁹ cells mL⁻¹. The concentration for each experiment was obtained following this method and adjusted by standard progressive dilutions.

2.3.2 Microbiological Analysis. Analysis in triplicate taken from both, treated (taken at the end of every hydraulic cycle) and untreated samples at 10⁷, 10⁴ and 10⁶ E. coli cells mL⁻¹ concentrations were compounded by inoculating 0.1mL in each Petri dish (90 mm diameter) containing LB agar through a spread-plate technique. Once labeled, the inoculated dishes were incubated overnight at 310.15 K for 86,400 s. After incubation period, the number of colonies from the initial cell concentration and the treated suspension was estimated as the number of surviving cells assuming that every viable bacteria results in the formation of a colony. Colony forming units (CFU) were enumerated, and concentrations per unit volume (CFU mL⁻¹) were determined by direct plate counting method.
2.4 Ozone Concentration Measurements

Ozone is one of the reactive chemical species generated by plasma in air/oxygen presence, which is able to produce important biological effects. By means of a probe connected to an ozone monitor (model 460L Teledyne Instruments), it is possible to measure the concentration (in ppm) of residual ozone generated during the discharge at the outlet of gaseous chemical species (see figure 1, point 4). The obtained data was processed on computer software in order to get a graphical interface where ppm concentration versus time is given.

2.5 Scanning Electron Microscopy (SEM) applied to norprene tubing

15 mL in volume of distilled water were introduced inside the reactor vessel and PDBD were applied during 600 s. Then, the water was transferred to a glass and a piece of norprene tubing was inserted inside until a period of 6 days was completed. SEM analysis was carried out in triplicate using a Scanning Electron Microscope JSM-5900 at 20 kV and magnifications \times 500 with the aim to determine if norprene is an acceptable material to be used as transport medium for fluids when PDBD process is applied.

3. Results and Discussion

The representative behavior of a discharge limited by a dielectric material is shown in figure 2, through voltage and current waveforms developed by a PDBD reactor during the treatment applied to inoculated water with \textit{E. coli} bacteria. According to Eliasson and Kogelschatz [13] and Rodríguez \textit{et al.} [14], once ionization occurs in the discharge gap, the charge is accumulated in the dielectric causing a limited streamer formation which is reflected in the formation of peaks, entailing to the reduced amount of transported charge into the medium as well as to the entire electrode area. The energy consumption per pulse during the experiment was obtained through the time-integral of the voltage-current product from the waveforms mentioned before, and it corresponds to 134.3 mJ pulse\(^{-1}\); this is bigger than those reported by Hernández \textit{et al.} [11], Rodríguez \textit{et al.} [12], which corresponds to \(\sim100\) mJ pulse\(^{-1}\). This variation could be attributed to differences of environmental conditions under experiments were carried out, geometry and internal surfaces of the electrodes and dielectric, and gas quality.

![Figure 2](image)

\textbf{Figure 2.} Voltage and current waveforms delivered by the PDBD reactor.

Results of the elimination efficiency of \textit{E. coli} bacteria in a recirculation system, where a PDBD reactor was coupled operating under steady and laminar flow conditions at 293.15 K and 103 kPa, carried out in presence and absence of oxygen gas are presented in figure 3. The required time for every fluid cycle corresponds to 419 s.

In figure 3 are depicted the results obtained in the bounded experimental period of time, at \(10^3\) CFU mL\(^{-1}\) \textit{E. coli} concentration. A reduction of \(>2\) log-orders was attained in presence of oxygen gas and
recirculating the fluid 12 times in the system; when the process occurs without gas flow, only <1 log-order bacterial concentration reduction was obtained. In the latter case, 2 additional fluid cycles were applied, obtaining a slight decrease of 1 log-order with respect to the initial concentration. In the case of 10⁴ CFU mL⁻¹ E. coli concentration, with the treatment applied during 18 cycles without oxygen, a reduction of <2 log-orders was obtained; this result was increased considerably at >3 log-orders through oxygen gas injection inside the system. When the treatment was applied at 10⁶ CFU mL⁻¹ E. coli concentration, it was registered a reduction over 3 log-orders in presence of oxygen in 22 fluid cycles; while the gas flow was absent, the elimination efficiency decreased to ~0.5 log-order in the same period of treatment. Therefore, as bacteria concentration increases, the number of required fluid cycles is higher; when the oxygen gas injected inside the system, log-orders decrease of E. coli concentrations is more significant, this effect is especially more evident at high concentrations.

The established experimental conditions in this work involve the oxygen gas injection inside the system in order to promote a mixture of liquid-gas fluids inside the reactor vessel and benefit the production of ozone. Figure 4 displays obtained behaviors of different oxygen gas flows injected inside the reactor. In all cases, it was observed that ozone concentration increased significantly during the first seconds of the process, later a constant tendency of the ozone concentration with respect to time occurred, indicating a saturation. At 8.3 mL s⁻¹, the ozone concentration is higher than the other proposed oxygen flows; it indicates that over >8.3 mL s⁻¹, the ozone concentration drops considerably due to an excess of oxygen gas, which was not used adequately to benefit the production of ozone.

According to the ozone monitoring during the treatment process applied to water without oxygen injection, it was generated 9.2 ppm while with oxygen 114.4 ppm. Increments in the efficiency of
elimination process of *E. coli* bacteria by the oxygen injection at $10^3$, $10^4$ and $10^6$ CFU mL$^{-1}$ concentrations are in the range of 3.5% to 28.5%. It could be attributed to the bacteria-discharge interaction during the included steps in recirculation process such as: flowing of the fluids at countercurrent mode, liquid flow sparging inside the reactor vessel and generating bubbles during the gas flow injection; some of the latter conditions mentioned are in agreement with those proposed by Glaze [16] in order to benefit the mass transfer or reactive chemical species such as ozone into the aqueous phase. Additionally, these results are in accordance with observations performed by Chen *et al.* [17] and Hernández *et al.* [11], who emphasized on the importance of ozone existence as an important chemical species able to cause damage in cytoplasmic membrane deleting the bacteriological activity.

Due to the temperature is considered a factor through the process efficiency could be modified, a cooling system was added to the reactor to maintain a stable temperature (293.15 K) during the treatment; it is in accordance with Gottschalk *et al.* [18], Ingraham [19] who verify that an increase in the temperature involve a decrease in the solubility of ozone and modifies its reaction rate.

Another important factor evaluated in this study corresponded to the norprene tubing used during the process for the liquid transport. Respectively, in table 1 and figure 5 are showing the analytical results and micrographies obtained by SEM before and after the treatment applied to water to eliminate *E. coli* bacteria.

According to the results, norprene tubing exhibited a slight variation in its composition when the elimination process of *E. coli* bacteria was applied; in the case of carbon and oxygen, it was observed an increase of 0.06% and 2.14%, respectively. On the contrary, identified elements in smaller percentages, such as: chlorine, aluminium, silicon and sodium decrease significantly; the last three elements were reduced so that they could not be detected by SEM. Figure 5(a) shows a micrography of norprene tubing before the treatment application; in it is observed a rugose structure due to many
disarranged pellets putting on it, which after the treatment (see figure 5(b)), adopted a rigid structure
without visible pellets over its surface and an appearance of humid film. In agreement with Askeland
and Phulé [20], to be norprene tubing a polymeric material, there exist dissolution of its components in
water during a prolonged exposure, carrying to the loss of them and weakening its structure.

Attention should be paid to the dissolution of chlorine in water, which contributes to the production
of chlorine, hexachlorobenzene, heptachlor and heptachlor epoxide and methoxychlor, currently
regulated by official laws due to their health risk. On the other hand, little increments of carbon and
oxygen, indicate a phenomenon of deposition on the tubing internal surface, as a result of the reactions
during the process. Additionally, norprene tubing surface could allow lodging and development of
microorganisms from one experiment to other; nevertheless, this possibility is eliminated due to it is
an autoclavable material.

Conclusions
A PDBD reactor coupled at a recirculation system, which includes a peristaltic pump type and a
cooling system, was designed and constructed to operate under steady and laminar flow conditions at
293.15 K and 103 kPa, 25-30 kV high voltage, 500 Hz frequency and 30 μs width, with the aim to
eliminate E. coli bacteria at $10^3$, $10^4$ and $10^6$ CFU ml$^{-1}$ concentrations found in water contaminated
with those microorganisms. Results indicate an elimination efficiency over 0.5 log-order when the
process was carried out in absence of oxygen; while >2 log-orders when oxygen gas was injected
inside the reactor during the treatment. As mentioned above, when bacteria concentration increases,
the number of required fluid cycles is higher; when the oxygen gas injection is carried out inside the
system, log-orders decrease of E. coli concentrations is more significant. Along the recirculation
process, experimental conditions including: flowing of the fluids at countercurrent mode, liquid flow
sparging inside the reactor vessel and generating bubbles during the gas flow injection, promote an
increase on the efficiency of the system. In the case of ozone production by oxygen gas, the efficiency
increased in the range 3.5% - 28.5% when injected 8.3 mL s$^{-1}$, flow which was determined in this
study as the optimum to produce ozone that contributes to eliminate E. coli bacteria.

Additionally, in this work was determined that norprene is an acceptable material to be used as
transport medium for the fluids, when PDBD process is applied to water in order to eliminate E. coli
bacteria; above mentioned is due to small detected changes by SEM on its atomic percentages
corresponding to main components such as carbon (+0.06%), oxygen (+2.14%) and chlorine (-0.05%)
and, on its internal surface, which after the treatment showed a more rigid surface. Additionally,
norprene tubing is autoclavable; therefore it is possible avoiding the lodging and development of
microorganisms inside it between experiments.

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