Plasma activated water bubbles: Characterization and assessment of desiccated *Salmonella* inactivation efficacy

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Abstract: This study investigated the antibacterial effectiveness of plasma activated water bubbles (PAWB) and plasma activated hydrogen peroxide water bubbles (PAHP-WB) against the desiccation survival of *Salmonella* Enteritidis FUA1946 on stainless steel. The pH, oxidation reduction potential, important reactive oxygen and nitrogen species in PAWB and PAHP-WB were determined. The effect of desiccation on the survival of *Salmonella* was dependent on the type sanitaizer and the process conditions.

Keywords: plasma activated water bubbles, hydrogen peroxide, *Salmonella*, desiccation, stainless steel

1. Introduction

Plasma Activated Water Bubble (PAWB) is a novel technology with significant potential for surface disinfection. It has the ability to increase the efficiency of mass transfer and the dissolution of plasma reactive species from the gas to liquid phase, thus making it a promising option for surface disinfection. Increased surface area to volume ratio and enhanced solubility of reactive oxygen and nitrogen species can enhance their antimicrobial effectiveness. PAWB is more sustainable and effective than conventional disinfection technologies since it is more environmentally friendly and produces no toxic byproducts. This study focussed on the characterization and applications of PAWB technology for the inactivation of desiccated *Salmonella*.

2. Materials and Methods

This research aimed to investigate the impact of specific design parameters of a bubble spark discharge reactor on the characteristics of Plasma Activated Water Bubbles (PAWB) and its microbial inactivation efficacy. The study was divided into three parts, with the first part focusing on understanding the effect of the selected design parameters on PAWB. The second part of the research focused on using PAWB technology to inactivate desiccated *Salmonella enterica* on stainless steel coupons. In the third part, a PAWB reactor equipped with a 3D printed coupon holder was designed to continuously treat the coupons contaminated with desiccated *Salmonella*.

Salmonella Enteritidis FUA1946 was used as a model pathogen. Stainless steel coupons (SSCs type 304) with the dimensions of (1 × 1 × 0.1 cm) were used as test surfaces. The experimental protocol consisted of two distinct desiccation conditions. In the first set of experiments, the efficacy of the *S.* Enteritidis FUA1946 cells inoculated on SSCs and dried in the biosafety cabinet was assessed. In the second set, referred to as "equilibration challenge assays", *Salmonella* cells were pre-adapted to the equilibration stress. Similar to the first set of experiments, the SSCs were inoculated with the same concentration of the *Salmonella* suspension and air-dried in the biosafety cabinet at 22–25 °C for 1 h. The inoculated coupons were exposed to 33% relative humidity (RH) and were equilibrated for 5 days.

Plasma activated water bubbles (PAWB): Fifty milliliters of PAWB were produced utilizing a bubble spark discharge reactor with atmospheric-pressure air (1 slpm) as the carrier gas. Plasma process parameters of 160 V, 66 µs duty cycle, and 1000 Hz were used. An ice jacket was utilized to prevent the water's temperature from rising excessively.

Plasma activated hydrogen peroxide-water bubbles (PAHP-WB): Plasma activated hydrogen peroxide water bubbles were prepared by activating 0.1 M and 1 M H_2O_2 for 10 min using the bubble spark discharge reactor based on initial experiments.

PAWB was produced for 10 min and 30 min followed by 1, 5, and 10 min of treatment periods. Similarly, PAHP-WB (0.1 M and 1 M) was produced for 10 min and treatment periods of 1, 5 and 10 min were employed. PAWB and PAHP-WB were investigated for their antibacterial activity against air-dried and air-dried and equilibrated *Salmonella* cells adhering to SSCs.

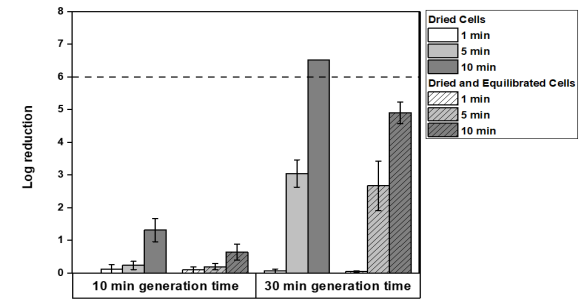
Plasma under water discharge characterization*:* The optical emission spectra (OES) of the excited species generated during the plasma discharge under water was measured using a spectrophotometer. The concentrations of the major reactive oxygen (ozone and hydrogen peroxide) and nitrogen species (nitrite and nitrate) was determined using CHEMetrics test kits.

Semi-continuous PAWB reactor with coupon holder: In order to simulate industrial conditions, a semi-continuous coupon holder was designed. Stainless steel coupons inoculated with the air-dried and equilibrated *Salmonella* were mounted on the holder and the PAWB was circulated under various combinations of plasma holding and flowing time. Similar plasma process parameters were employed as described above. The flow rate of the PAWB was 100 ml/min.

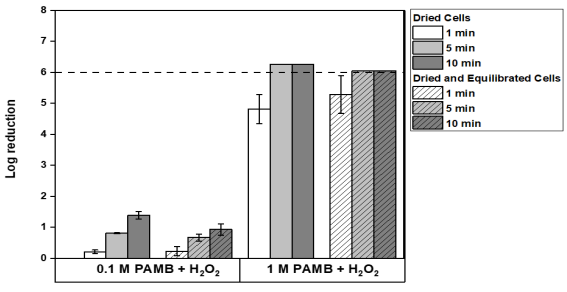
Effect of selected process parameters on plasma bubble spark discharge: To determine the effect of process parameters, *Salmonella* was inoculated to 50 ml of sterilized water and circulated through the semi-continuous PAWB reactor. The effects of selected air flow rates (0.5, 1, 2 and 4 slpm) and the distance of bubble opening from the bottom tip of high voltage electrode (1, 2, 3 and 4 cm) inside the bubble spark discharge reactor on RONS concentrations and *Salmonella* inactivation rates were determined.

3. Results and Discussion

Efficacy of PAWB and PAHP-WB: The PAWB generation time significantly ($p < 0.05$) increased the reduction of both the cell types (Fig. 1a). Significant interactions between the generation time and the treatment time against the reduction of *Salmonella* on SSCs was observed This was prominently observed for PAWB generated for 30 min, a 10 min treatment time reduced the air-dried cells to below detection limit of 1.3 log CFU/cm^2, in contrary the air-dried and equilibrated cells observed a lesser reduction of 4.90 ± 0.33 log CFU/cm^2.



(a)

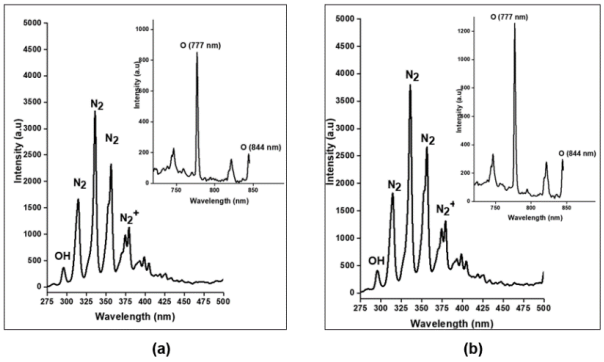


(b)

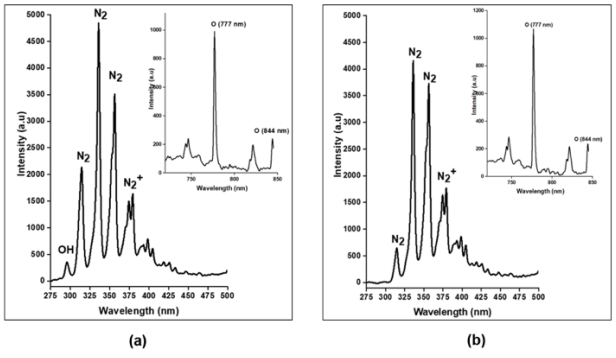
Fig 1. Sensitivity of *S.* Enteritidis FUA1946 on SSCs to (A) plasma activated water bubble (PAWB) and (B) plasma activated hydrogen peroxide water bubbles (PAHP-WB) generated for 10 min and 30 min. Bars represent mean ± standard deviation of three independent trials.

Increasing the concentration of H_2O_2 in the PAHP-WB from 0.1 to 1 M, significantly ($p < 0.05$) increased the reduction of both air-dried and air-dried and equilibrated *Salmonella* on SSCs (Fig. 1b). A 5 min exposure to 1 M PAHP-WB reduced both the cell types to below detection limit of 1.3 log CFU/cm^2. The desiccation time had no effect ($p = 0.2682$) on the reduction of *Salmonella* to PAHP-WB treatment.

Emission spectra observed prominent peaks of OH, N_2, N_2^+ in the UV region (280 – 400 nm). The intensity of the peaks increased with an increase in the PAWB generation time (Fig. 2 (I)). Significant O peaks were observed at 777 and 844 nm for PAWB generated for 10 min (Fig. 2 (Ia)). The intensity of the O peaks also increased with an increase in the PAWB generation time of 30 min (Fig. 2 (Ib)). Increasing the PAWB generation time resulted in significantly higher production of the ROS (O_3 and H_2O_2) and RONS (NO_2^- and NO_3^-). Overall, irrespective of the PAWB generation time and treatment time, air-dried *Salmonella* on SSCs were significantly ($p < 0.05$) more susceptible to the PAWB treatment as compared to the air-dried and equilibrated cells.



(I)



(II)

Fig 2. Optical emission spectra of (I) PAWB at (a) 10 min generation, and (b) 30 min generation, (II) PAHP-MB at (a) 0.1 M and (b) 1 M concentration, generated for 10 min.

The intensity of the O peaks observed at 777 and 844 nm were higher at 1 M PAHP-WB (Fig. 2 (IIb)) as compared to 0.1 M PAHP-WB (Fig. 2 (IIa)), however more intense peaks for N_2 spectra were observed for 0.1 M PAHP-WB.

Also, OH peaks were found to be missing with increasing the concentration of the PAHP-WB (Fig. 2 (II)).

The antibacterial efficacy of PAWB was attributed to the oxidative stress caused by the synthesis of short-lived ($OH^{\bullet}$, $NO^{\bullet}$, O_2^-, $OONO_2^-$, $ONOO^-$) and long-lived (NO_2^-, NO_3^-, H_2O_2, O_3) reactive species [1]. The concentration of RONS increased significantly and the pH of the experiment dropped from 7.1 to 2.64 and 2.66 after 10 and 30 minutes of PAWB production, respectively.

PAWB is composed of electrons, photons, charged particles, and reactive oxygen and nitrogen species; its efficacy can be amplified by combining it with H_2O_2 [2]. Plasma activation of H_2O_2 generates greater quantities of both short- and long-lived (HNO_2, NO_3, H_2O_2) reactive species, which can contribute to its enhanced inactivation efficiency (Fig. 1b). The high concentration of RONS produced by 1 M PAHP-WB, resulted in a higher reduction of dried *Salmonella* on SSCs.

***Effect of selected process parameters on plasma bubble spark discharge**:* The air flow rate significantly influenced the *Salmonella* Typhimurium inactivation in PAWB. For instance, air flow rate of 2 slpm in bubble spark reactor resulted in greater *Salmonella* inactivation compared to the other air flow rates used. However, the ORP, hydrogen peroxide, and nitrates in PAWB produced by 2 slpm were lower compared to the other tested air flow rates, showing the activity of additional antimicrobial reactive species. The distance of bubble opening from the bottom tip of high voltage electrode significantly influenced *Salmonella* inactivation in PAWB, however, the inactivation results did not correlate with the tested reactive species concentration and more research is required to understand the effect of other potential factors.

Semi-continuous PAWB reactor with coupon holder: There was a significant ($p < 0.05$) effect of treatment conditions on the reduction of air-dried and equilibrated *Salmonella*. Different hold and flow combinations were used for a total of 25 minutes of treatment time. Higher holding times of 10 min, 12.5 min, and 15 min observed a significantly similar reduction of the *Salmonella* cells. In contrary, decreasing the PAWB holding time and increasing the flow time dramatically increased the reduction of air-dried and equilibrated *Salmonella* on SSCs. (Fig. 3).

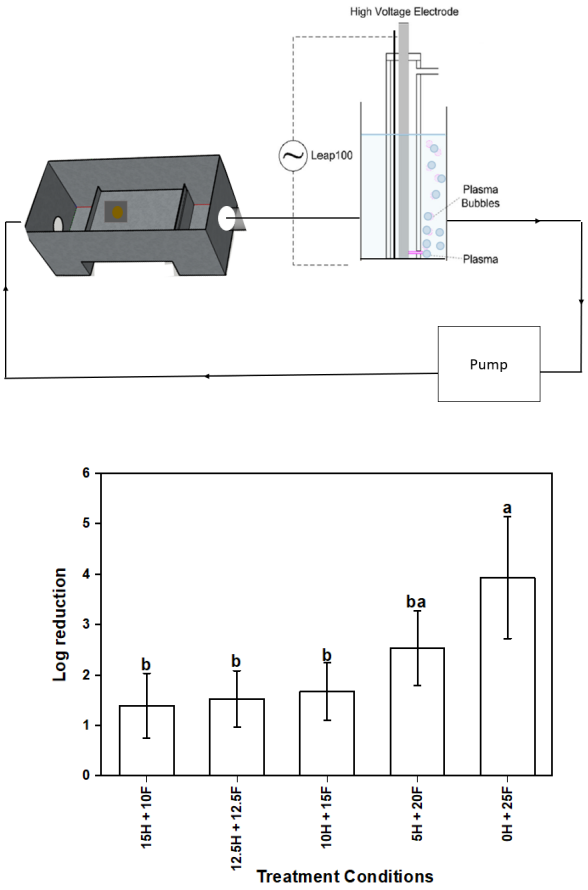


Fig 3. Inactivation of *S.* Enteritidis FUA1946 on SSCs using the semi-continuous PAWB reactor with coupon holder. White bars indicate the cells air dried for 1 h and equilibrated to an a_w of 0.33 for 5 days. Each point represents mean ± standard deviation of three independent trials. Treatment conditions used were 15 min hold + 10 min flow, 12.5 min hold + 12.5 min flow, 10 min hold + 15 min flow, 5 min hold + 20 min flow, 0 min hold + 25 min flow in the coupon holder.

4. Conclusions

Overall, this study demonstrated the potential for continuous production of PAWB and efficient reduction of desiccated *Salmonella* on stainless steel surface by this novel technology. Desiccation method significantly increased the resistance of *Salmonella* to PAWB, however, no effect of desiccation condition was observed when PAHP-WB was used.

5. References

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