Effect of DC micro-plasma treatment on bacteria in its vegetative bacteria

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Abstract. Argon gas plasma consists of discharge pattern of micro hollow cathode. The pattern is used to inactivate the two main kinds of bacteria. The Culture of Escherichia Coli (gram positive) and Staphylococcus aureus (gram negative) are subjected to the plasma exposure. The micro-plasma jet (MPJ) discharge was generated in the voltage range of 2-10 kV with an operating current of 20-35 mA. Under such conditions, a MPJ of approximately 1 cm visible length was produced. The power efficiency of this device was approximately 80%. The efficacy of MPJ to inactivate the bacteria is determined by observing colony forming unit (CFU) counts before and after plasma exposure. The Colony counter is used here to find the CFU/plate. The effect of plasma exposure is observed by varying the time at 15, 30, 60, 90, 120, 150 and 180 seconds. An efficient reduction in CFU is observed after plasma exposure, particularly for 120 seconds. Approximate 50% decay of both cultures is observed after treatment time of 120 seconds.

1. Introduction
Plasma can be categorized into two classes, namely thermal plasma and non-equilibrium or low-temperature plasma (LTP) [1]. In the non-equilibrium plasma, electrons have higher temperatures and gas molecules have moderate temperatures without any local thermodynamic equilibrium, which renders lower temperatures to the whole system [2]. Cold plasma can be defined as “partially or wholly ionized gas carrying myriads of highly reactive species like electrons, negative ions, positive ions, free radicals, excited or non-excited atoms and photons at around ambient temperature”. At atmospheric pressure, “cold plasma” is receptive to in-stabilities. By confining such plasmas to small dimensions has been given the reliability of atmospheric-pressure air plasma. Non-thermal micro plasma in DC discharges are generally created in closed discharge vessels using internal electrodes at relatively high potentials. These energies dissociate the gas molecules into a spark of ions, electrons, free radicals, charged/neutral gas molecules and other species which are active biologically and have potential to destroy microorganisms [3]. Low temperature DC-cold atmospheric pressure plasma consists of reactive species, which has attained considerable attention in biomedical application such as in wound healing, sterilization of heat sensitive reusable medical instruments or the surface.
modification of biocompatible materials [4]. Most of the “micro plasma” designs, such as plasma needles [9]. The atmospheric pressure plasma jets, several corona discharges and micro hollow cathode discharges are being developed for further applications in medical.

The properties of plasma can be altered by changing current, pulsed excitation time and voltage. A considerable improvement in tooth-whitening process, utilizing MPJ, is possible as compared to the conventional clinical methods [12]. The non-thermal RF-driven plasma jets of argon gas have been tested for antimicrobial treatment of heat sensitive materials. These jets can be operated at low powers, typical ranging from 10 W to 60 W. The highest reduction factor for viable bacteria is observed after direct plasma treatment of inoculated test strips [16]. It has been demonstrated by several authors that plasma treatment is an effective method due to production of UV radiations that can reduce the micro-organism at lower temperatures and even at ambient pressure up to 1 atm [8, 16].

In this study, non-thermal micro hollow cathode discharge (MHCD) design, based on clod plasma micro jet (PMJ), generated at atmospheric pressure [13]. Two main kinds of bacteria Escherichia Coli and Staphylococcus aureus were treated with PMJ. The samples were prepared and characterized in a petri dish for plasma exposure. A considerable reduction in the growth of these species is observed after the plasma exposure. The number of CFU were examined before and after plasma treatment.

2. Materials and methods

2.1. Production of micro-plasma jet

MHCD conception is used to generate an atmospheric pressure PMJ. The said structure comprises of a cathode with a micro hollow structure and an anode of copper rod. As the operating gas is propelled through this set up and a DC source is applied, a well-defined and concise PMJ can be produced in ambient air [6]. The appliance used here was developed built in Plasma Research Lab at the Department of Physics, University of Agriculture Faisalabad.

The opening of plasma jet nozzle was 2 mm. The copper anode was merged into bacterial media and powered with an external DC source. A hollow cathode was placed just above the media. A 2-20 kV DC supply was used generate PMJ for plasma exposure of bacteria. A blast resistance of 100 kΩ was placed between the power source and cathode to avoid circuit short. Compressed Argon gas was used as plasma precursor at a flowrate of approximately 2 SLM. The discharge current was measured in the range of 20-35 mA. Under such conditions, a PMJ of approximately 1 cm visible length was produced. The power efficiency of this device is approximately 80%. The temperature of the ionized gas was around 37 °C under atmospheric pressure.

2.2. Bacteria culture

Two most important bacteria cultures, namely Escherichia Coli and Staphylococcus aureus were treated with PMJ. The bacteria were grown and characterized in the Micro-biological lab at the University of Agriculture Faisalabad, Pakistan. These suspensions were grown in petri dishes and incubated for 24 hours to obtain the proper growth of bacteria. Plasma-induced deactivation of bacteria was observed for the specific stain of E.coli and Staphylococcus aureus [11]. The bacteria count, before and after plasma treatment, was carried out through CFU count. CFU is a way of finding colony forming units, as shown in figure 1. CFU/plate recorded for each petri dish before the treatment is given in table 1. The bacterial culture was carried out at room temperature. E.coli was gram positive whereas Staphylococcus aureus was gram negative.

| Bacteria                  | Culture Temperature | Gram stain |
|--------------------------|---------------------|------------|
| E.coli                   | 37°C                | Positive   |
| Staphylococcus aureus    | 37°C                | Negative   |

Table 1. Bacteria cultures preparation for treatment.
2.3. Plasma treatment of bacteria
To avoid contamination, the treatment with plasma is carried out on a laminar flow clean bench. The jet nozzle was place just above the bacteria suspension. The bacterial samples were treated for different periods of time, as given in table 2. The efficacy of PMJ to inactivate the bacteria is determined by observing CFU count before and after plasma exposure [13]. The cultures treated with plasma were placed in petri dishes as well as incubated and sealed in a box at 37 °C temperature nearly 20-24 hours. CFU count for each plate before and after the treatment allowed us to get information about plasma efficiency.

Table 2. A manual description of inactivation of bacteria cultures.

| Exposure time (s) | CFU/plate (E.coli) | CFU/plate (Staphylococcus) |
|-------------------|-------------------|---------------------------|
| 0                 | 81                | 185                       |
| 15                | 77                | 180                       |
| 30                | 79                | 168                       |
| 90                | 65                | 132                       |
| 120               | 45                | 92                        |
| 150               | 44                | 90                        |
| 180               | 42                | 87                        |

3. Results and discussion
Initially, the impact of air on bacteria deactivation was observed without any plasma exposure. No particular decline of microbes was observed due to air flow. CFU numbers were assessed for both treated and untreated area of the petri dish. Due to the various coatings and layers, surrounding the genetic nucleus, spores were harder to inactivate [5]. The findings are reported in figure 2. All the microbes deactivated in the region exposed to plasma. With the passage of time, the bacteria within the unexposed parts also deactivate as shown in figure 2 (b).

While observing CFU for said two bacteria cultures, there was reasonable decrease in CFU count after plasma exposure. The plasma exposure is studied for cultures by using six different treatment time intervals ranging from 15 to 180 seconds. For smaller treatment times, E.coli CFU count decreases in small ratio but for treatment time of 120 seconds, the CFU count falls from 81 to 45. Similarly, Staphylococcus aureus CFU count decreased from 185 to 92 after treatment time of 120 seconds. There was a gradual decrease in CFU count of both types of bacterial below the exposure time of 120 seconds. A noticeable decay of CFU count was observed at the exposure time of 120 seconds [7]. It reveals that PMJ is effective technique for reducing CFU of bacteria if the exposure time is properly tuned. Table 2 provides information on CFU count over plasma exposure time. After 180 seconds CFU count of E.coli and Staphylococcus aureus was decreased to 42 and 87, respectively.
Figure 2. Scattering configurations of remaining CFUs on the petri dish after PMJ treatment for 180 s: (a) S. aureus and (b) E.coli.

Although CFU counting was carried out using a colony counter, the pictures of CFU in petri dishes can be taken by using a high resolving camera. Picture sin figure 3 reveal a gradual decrease in CFU count with increasing the treatment time [14, 15].

Figure 3. Treated bacteria cultures of the S. aureus for the different treatment time.

The bottom row in figure 3 shows the photographs of untreated bacteria and the top row shows the photographs of corresponding treated bacteria. The graphical representation of the observed response of CFU to the plasma exposure is given in figure 4 and figure 5. These plots clearly show a decreasing trend of CFU count over exposure time. For both bacteria, a sudden decrease in CFU count is observed after plasma exposure time of 120 seconds.
Figure 4. A graphical decay in CFU count of Staphylococcus aureus.

Figure 5. A graphical decay in CFU count of E.coli.

4. Conclusion
A dc atmospheric Argon gas cold plasma micro jet was used to deactivate two types of bacteria in their vegetative state. The presented plasma treatment model proved as an effective method for inactivation of bacteria in their vegetative state. Two main classes of bacteria cultures namely E.coli and Staphylococcus aureus were treated with plasma to obtain the clear effect of plasma exposure on microbes. E.coli CFU count decreased from 81 to 45 after treatment time of 120 seconds. Similarly, Staphylococcus aureus CFU count decreased from 185 to 92 after same treatment time. A noticeable decay of CFU count was observed at the exposure time of 120 seconds. After 180 seconds, CFU count of E.coli and Staphylococcus aureus was decreased to 42 and 87, respectively. It reveals that PMJ is an effective technique for reducing CFU of bacteria if the exposure time is properly tuned.

References
[1] Conrads H and Schmidt M 2000 Plasma Sourc. Sci. Tech. 9 441.
[2] Haertel B, Von Woedtke T, Weltmann K D and Lindequist U 2014 Biomol. Ther 22 477.
[3] Moselhy M, Petzenhauser I, Frank K and Schoenbach K H 2003 J. Phys. D: Appl. Phys. 36.
[4] Ehlbeck J, Schnabel U, Polak M, Winter J, Von Woedtke T, Brandenburg R and Weltmann K D 2010 J. Phys. D: Appl. Phys. 44 013002.
[5] Dobrynin, D, Fridman G, Mukhin Y V, Wynosky-Dolfi M A, Rieger J, Rest R F and Fridman A 2010 IEEE T. Plasma Sci. 38 1878.
[6] Deng X L, Nikiforov A Y, Vanraes P and Leys C 2013 J. Appl. Phys. 113 023305.
[7] Feng H, Sun P, Chai Y, Tong G, Zhang J, Zhu W and Fang J 2009 IEEE T. Plasma Sci. 37 121.
[8] Laroussi M and Leipold F 2004 Int. J. Mass Spectrom. 233) 81.
[9] Laroussi M, Tendero C, Lu X, Alla S and Hynes W L 2006 Plasma Process. Polym. 3 470.
[10] Shen J, Sun Q, Zhang Z, Cheng C, Lan Y, Zhang H and Chu P K 2015 Plasma Process. Polym. 12 252.
[11] Liu F, Sun P, Bai N, Tian Y, Zhou H, Wei S and Fang J 2010 Plasma Process. Polym. 7 231.
[12] Sun, P, Pan J, Tian Y, Bai N, Wu H, Wang L and Fang J 2010 IEEE T. Plasma Sci. 38 1892.
[13] Hsu D D and Graves D B 2003 J. Phys. D: Appl. Phys. 36 2898.
[14] Shen J, Sun Q, Zhang Z, Cheng C, Lan Y, Zhang H and Chu P K 2015 Plasma Process. Polym. 12 252.
[15] Brugger S D, Baumberger C, Jost M, Jenni W, Brugger U and Mühlemann K 2012 Plos One 7 e33695.
[16] Brandenburg R, Ehlbeck J, Stieber M, Woedtke T V, Zeymer J, Schlüter O and Weltmann K D 2007 Contrib. Plasma Phys. 47 72.