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ARTICLE

DNA Reduction of Waterborne *E.coli* by Underwater Capillary Discharge

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Abstract: *Escherichia coli* (*E. coli),* (190CRC) is one of the most common waterborne pathogens. It exists in drinking water and most of the water diseases are associated with it. The permanent inactivation of *E.coli* from water requires its DNA distortion, that makes it ineffective for regeneration. The underwater plasma discharge in capillary tube induces reactive oxidant species {$0H^{\cdot}$ radicals, Ozone (O_3), hydrogen peroxide (H_2O_2) and reactive oxygen (O)}. Ultraviolet (UV) radiations are most important shock waves that play a vital role for complete degradation of *E.coli* from water. In the current study, the effects of plasma for different oxygen injection rates and applied voltages on DNA distortion of *E.coli* have been reported. The bactericidal tests including DNA and protein leakage of plasma-treated water showed complete distortion of *E.coli* DNA structure and sterilization of water from bacteria.
Keywords: Capillary discharge, *E.coli*, DNA, Protein leakage, Sterilization.

1. Introduction

Plasma discharge in aqueous systems has attracted the attention of researchers around the globe due to its novel applications in water purification, waste treatment, industrial, agricultural, nanotechnology, semiconductors, material processing and antibacterial activities [1-5]. The required chemical reactions for such applications can be enhanced by plasma-induced ultraviolet (UV) radiations, shock waves, reactive oxidant species (ROSs) as well as charged particles [6]. In recent years, many studies on plasma types, like corona, arc and spark discharges within water and on the surface of water, have been conducted. Different (AC, DC and RF) power sources and electrode assemblies; i.e., pin-pin, pin-plate and plate-plate configurations, have been tested for performance judgement [7-11]. Capillary discharge has proven to be an advantageous technique as compared with the previous approaches in perspective of power consumption for large-volume flowing-water treatment, device portability, system cost and discharge controllability [12-15]. The capillary-produced plasma is supposed to be uniform with average electron density rising to $1x10^{19}cm^{-3}$ and average electron temperature around 2eV [16]. Such uniform plasmas are of great interest for advanced medical, biological and agricultural applications. In this study, we have implemented underwater capillary discharge technique and reported the results for *E.coli* DNA distortion for various values of applied voltages and oxygen injection rates.

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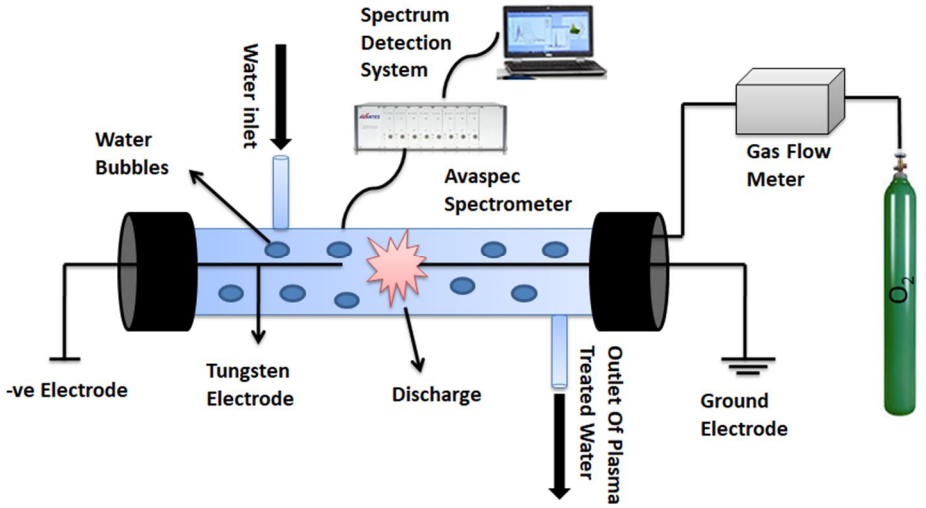
2. Materials and Methods

2.1 Experimental Details

Plasma discharge was initiated in a quartzd capillary tube (outer diameter =4mm, inner Diameter = 2mm, inner, thickness= 1mm). Alternating voltage was applied across two tungsten electrodes (diameter =0.5mm), through a high-frequency (758 kHz) plasma generator CTP-2000S. Fig. 1(a) shows an illustration of the experimental setup, while Fig. 1(b) shows a visual view of capillary discharge. The separation between two electrodes where discharge occurred was kept at 5mm. The water flow was kept at 0.1 L/min, while the flow of oxygen was controlled by a mass-flow controller TELEDYNE-500P and injected by a medical syringe to initiate water bubbles and gas channels inside the capillary. This drastically reduced the required power to initiate the discharge and induced a high concentration of reactive oxidant species useful for disinfection. Emission spectrum was recorded by an Avantes multichannel spectrometer (having an optical resolution of 0.06nm-1.3nm), to determine the concentration of reactive oxidant species ($OH^{\cdot}$ radicals, O_3, H and O). The treated water was tested by the micro-well dilution method, readily after plasma treatment. Later, it was periodically examined after every 12 hours up to 48 hours to detect whether there is a regeneration of *E. coli* in water with the passage of time and an impact of plasma treatment on the geometry of bacterial colonies.

(a)



(b)

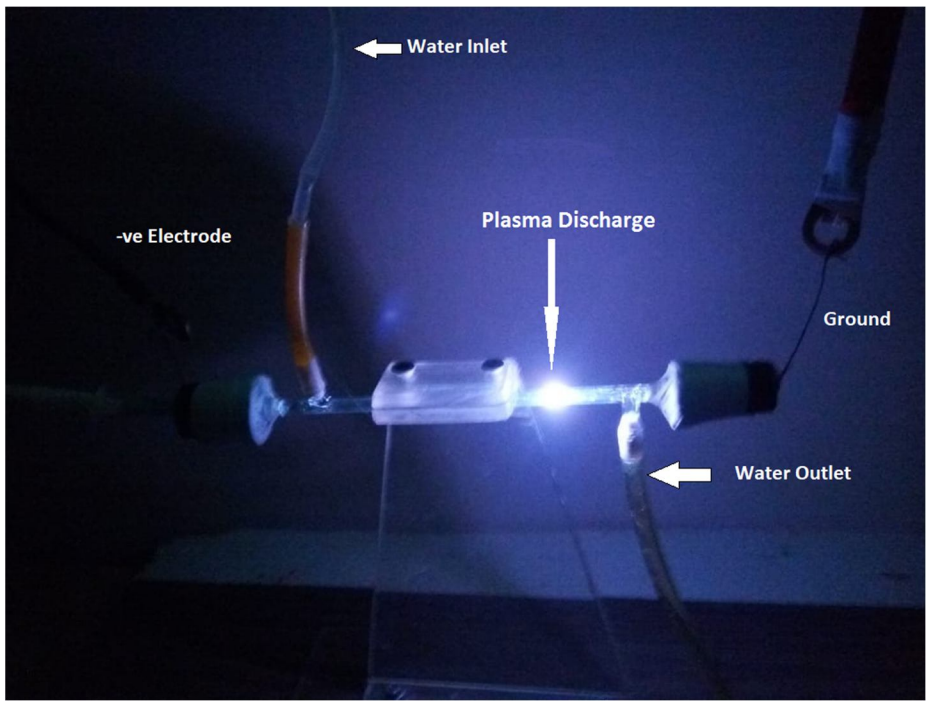


FIG. 1. (a) Schematic diagram of capillary discharge. (b) Visual view of capillary discharge.

2.2 Bacterial Strains Used and Growth Conditions

Multidrug-resistant strains isolated from clinical *Escherichia coli* (190 CRC) samples (resistant to ampicillin, erythromycin, ciprofloxacin, nalidixic acid, streptomycin, sulfamethoxazole –trimethoprim, gentamycin) were collected from Microbiology and Public Health Lab, COMSATS University, Pakistan. *Escherichia coli* was revived on Luria-Bertani (LB) broth (Sigma-Aldrich, Ireland, Ltd.) agar plates. For each set of experiments overnight (16 to 18 hrs), bacterial cultures were grown in Lauria broth at 37°C and used for investigations.

2.3 Bacterial Cell Survival Assay

Overnight, culture of each bacterial strain (~10^7 CFU) was used to inoculate autoclaved distilled water to make water suspensions. These suspensions were then used to prepare different groups of experimental design (with and without treatment) and were then exposed to plasma for different treatment times. Each sample was then serially diluted, spot-plated onto LB agar and the plates were incubated at 37°C for 24 hrs. The number of colonies on each plate was counted and the colony-forming units per ml (CFU/ml) were calculated using the following formula:

$$\text{Colony} - \text{forming units(CFU/ml)} = \frac{\text{No.of colonies X Dilution factor}}{\text{Volume of culture plated}}. \quad (1)$$

The killing % overall inactivation efficiency of plasma was calculated using the following formula:

$$\text{Killing \%} = \left(1 - \frac{\text{No.ofCFU/mlin treated samples}}{\text{No.ofCFU/mlin untreated control samples}}\right) * 100. \quad (2)$$

Untreated water suspension and un-inoculated water were used as positive and negative controls.

2.4 DNA and Protein Leakage Assay

Nightlong grown bacteria cultures were exposed to plasma. The samples were centrifuged at 14000 RPM and the supernatant was moved through a 0.22 µm (TS). By using Nano-drops, the DNA measurement was performed and Bradford assay was used for the perception of protein in the supernatant (Sampathkumar et al., 2003). Leakage index was calculated by using the following formulae:

$$\text{DNA Leakage Index} = (TS_{\text{O.D 260}} - UTS_{\text{O.D 260}})/TTS_{\text{O.D 260}} \quad (3)$$

$$\text{Protein Leakage Index} = (TCS_{\text{O.D 595}} - UTS_{\text{O.D 595}})/TTS_{\text{O.D 595}} \quad (4)$$

Untreated samples (UTS) and Triton X-100 (TTS)-treated cells were used as negative and positive controls. All experiments were performed in triplicate and repeated thrice.

3. Results and Discussion

Visual, biological and statistical methods were used to investigate and report the DNA distortion efficiency of capillary discharge.

3.1 Plasma Emission Spectrum

Due to intrusive nature, optical emission spectroscopy (OES) is a fundamental diagnostic tool, used for determining the plasma composition and concentration of highly reactive oxidant species, which play a vital role in the plasma sterilization process.

Typical emission spectrum of underwater capillary discharge is presented in Fig. 2. The spectrum reveals the existence of highly reactive oxidant species ($OH^{\cdot}$ =309nm, UV radiations = 240-400nm, Reactive oxygen = 777 and 844nm and Ozone =883nm).

After discharge occurrence, the dissociation of O_2 atomic oxygen was formed, while due to collision of electrons and water molecules, reactive hydrogen (H_α = 656 nm, H_β = 444nm) was formed as mentioned in the following reactions [17]:

$$O_2 + e \rightarrow O^+ + O + 2e \quad (5)$$

$$O_2 + e \rightarrow O^- + O \quad (6)$$

$$H_2O + e \rightarrow H + OH + e \quad (7)$$

Ozone (O_3) having a redox potential of 2.07 V was generated when diatomic oxygen gets converted into atomic ions (O^+) by plasma electrons and oxygen radicals and later, these radicals undergo three –body reaction to generate Ozone given as [17, 18, 19]:

$$O + O_2 + M \rightarrow O_3 + M \quad (8)$$

Ozone-produced $OH^{\cdot}$ radicals react with (OH^-) and H_2O_2 as given in the following reactions :

$$O_3 + 2OH^- \rightarrow OH^{\cdot} + HO^*{}_2 + O_2^{\cdot -} \quad (9)$$

$$O_3 + H_2O_2 \rightarrow OH^{\cdot} + HO^*{}_2 + O^{\cdot}{}_2 \quad (10)$$

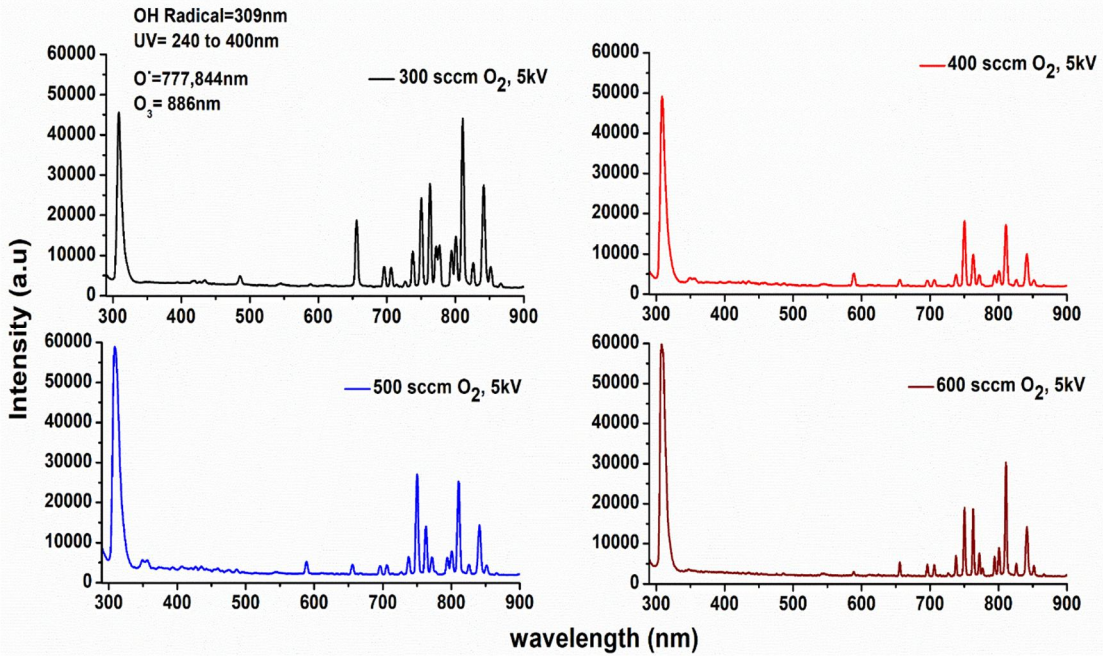


FIG. 2. Typical plasma emission spectrum.

After generating plasma, the transitions (excitations to the ground states) of atomic and molecular species cause emission of UV radiations, observed from the emission spectrum. The emission spectrum represents variation in UV radiation and $OH^{\cdot}$ radical densities as a function of applied voltage for different flow rates. Due to increasing applied voltage and oxygen rate, the power of discharge pulses increased prominently. The production of $OH^{\cdot}$ Radicals and UV radiation also increased and a much brighter discharge spectrum was observed. Since $OH^{\cdot}$ radicals have a short half-life (10^{-8}s), the decay of $OH^{\cdot}$ radicals further triggers the production of other reactive species, such as O_3. The electron temperature rises with the increase in applied voltage, which ultimately enhances the production rates of $OH^{\cdot}$ radicals and UV radiation. Similarly, with the increase in gas-flow rate, electrons will effectively participate in collisional processes, resulting in the creation of more reactive species like $OH^{\cdot}$ radicals and increasing UV radiation intensity.

3.2 *E.coli* Inactivation by Plasma Discharge

E.coli colonies after plasma treatment under different experimental conditions are shown in Fig. 3 in the form of typical images of LB agar plates, while the graphical illustration of all *E.coli* CFUs is presented in Fig. 4. After plasma treatment, at 3Kv, the CFU was reduced from 88 to 2 at 300sccm (standard cubic centimeter per minute) and 600sccm; at 4Kv, the CFU was reduced from 185 to 1 at 300sccm and 600sccm; at 5Kv, the CFU was reduced from 290 to 5 at 300sccm and 600sccm; at 6Kv, the CFU was reduced from 61 to 10 at 300sccm and 600sccm; at 7Kv, the CFU was reduced from 278 to 66 at 300sccm and 600sccm.

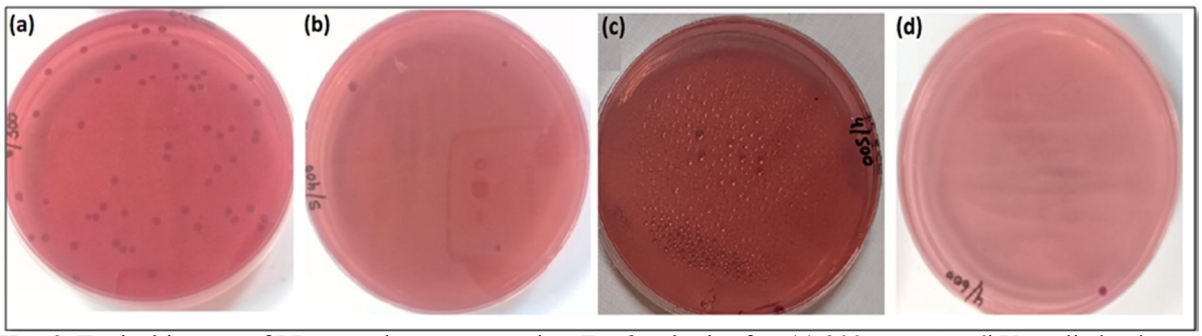


FIG. 3. Typical images of LB agar plates representing *E.coli* colonies for: (a) 300 sccm O_2, 6kV applied voltage. (b) 400 sccm O_2 5kV applied voltage. (c) 500 sccm O_2, 4kV applied voltage. (d) 600 sccm O_2, 4kV applied voltage.

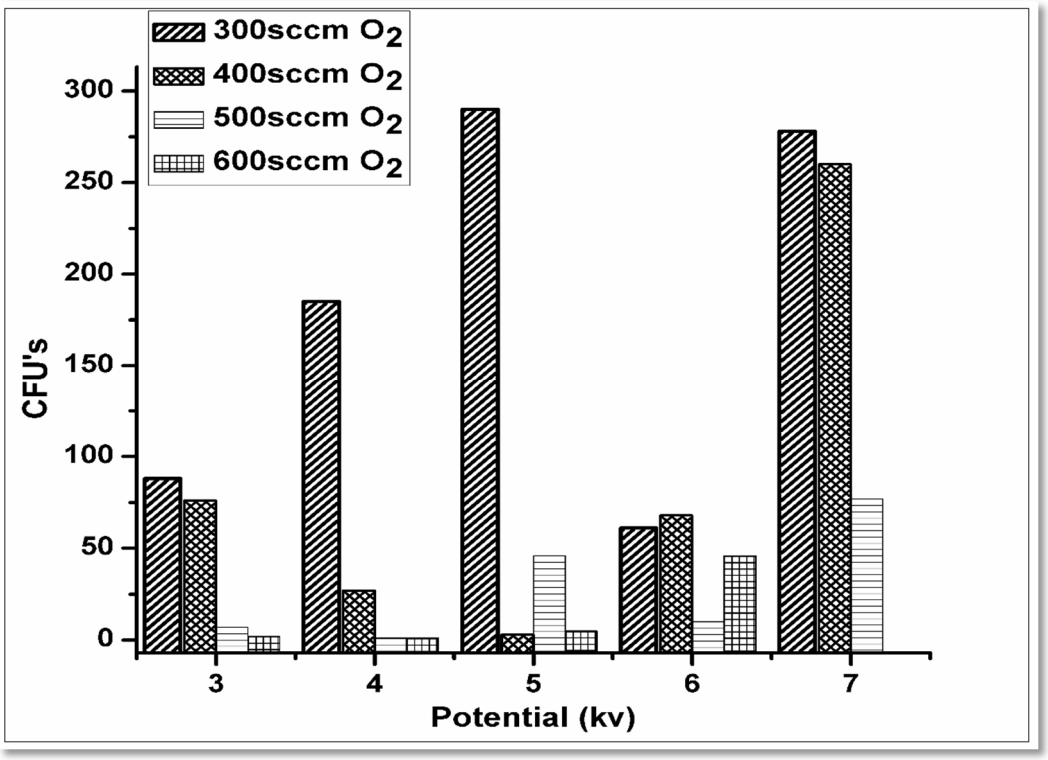
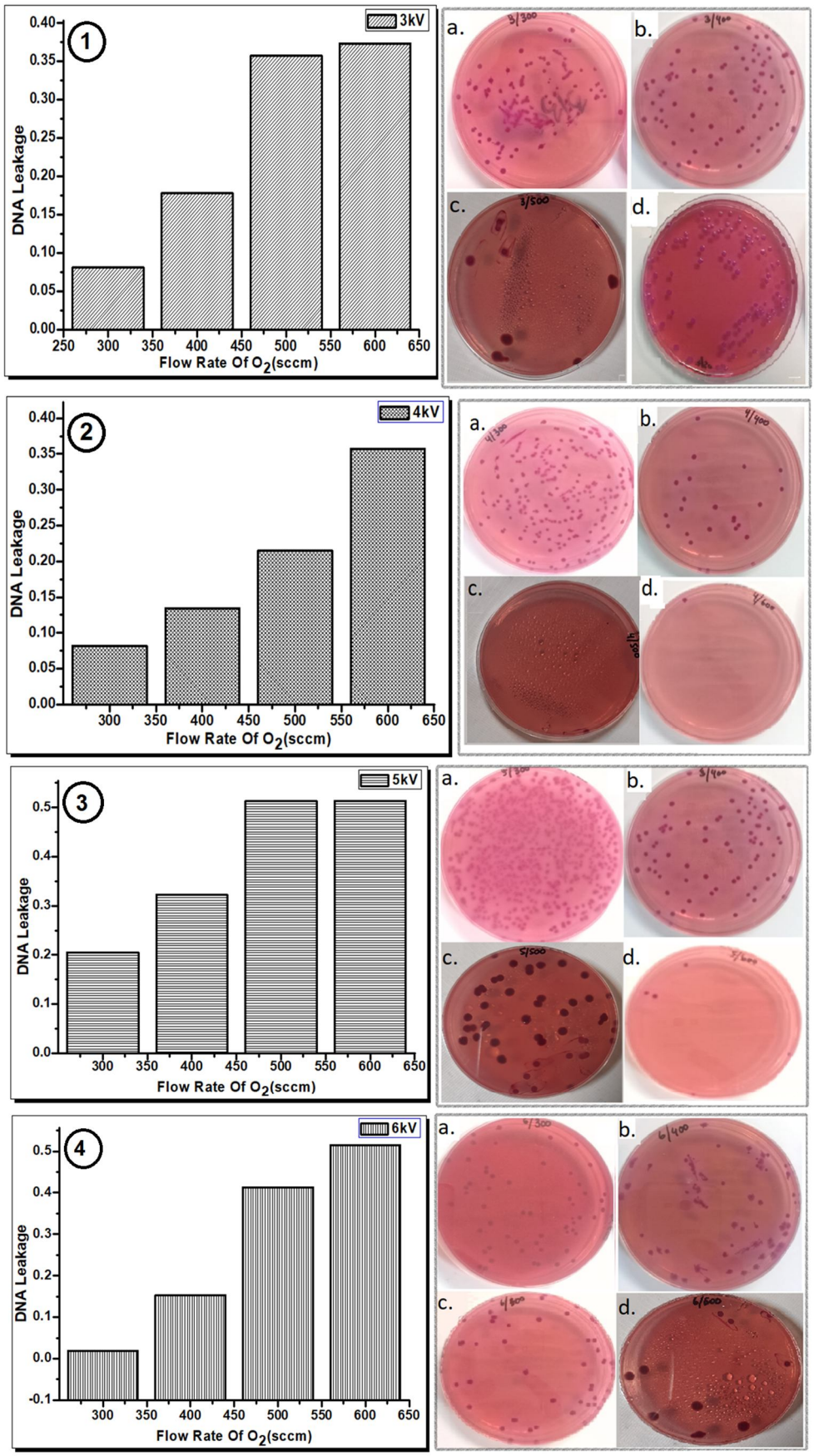


FIG. 4. CFUs of *E.coli* under different oxygen injection rates at various applied voltages.

Oxygen shows a remarkable influence on the inactivation of *E.coli* due to its potential resistance for the growth of bacterial cells. Increased oxygen injection rates encourage greater leakage of ROS from respiratory chain, which affects the DNA and metalloenzymes. This in turn leads towards mutagenesis and impaired growth. The $OH\cdot$ radical, UV radiations and shock waves have participated mainly in the inactivation of *E.coli*. They interact directly with bacteria in water, destroy the DNA structure and disable mutation. Shock waves also to increase the exposure of micro-organisms inactivation by scattering the colonies in liquid.

3.3 Deoxyribonucleic Acid (DNA) Analysis and Protein Leakage Tests

Proteins are important bio-molecules which play an important role in balancing bacterial cell physiological activity. The plasma treatment-induced DNA leakage and protein leakage through membrane of *E. coli* cell are represented in Fig. 5 and Fig. 6, respectively. The DNA damage of isolated nuclei is dependent on the concentration of oxygen. Protein concentrations were found to decrease with the increase in oxygen flow rate, which in turn damages the DNA of *E. coli*. The protein and DNA of gross untreated *E.coli* sample decreased to 0.413µg/mL and 0.011, respectively after 600 sccm at 4kV. DNA and protein leakage leads to bacteria killing by inhibiting antioxidant machinery that damages the membrane protein as well as a cascade of DNA repair. Being the heredity material, DNA is a very important component of the cell and is comprised of a double helix structure. By increasing the oxygen injection rate, the concentration of reactive species also increases, which in turn elevates the rate of DNA and protein leakage. Underwater plasma discharge multiple reactive species ($OH\cdot$, O_2, RO, RH and UV) were involved in DNA distortion. Most of the DNA damage was caused by ionizing radiations mediated by hydroxyl radical ($OH\cdot$) and quintessential reactive oxygen species. Such distortion involves strand splits, caused by the hydroxyl radical by abstracting a deoxyribose hydrogen atom. The presence of UV radiation and other genotoxic chemicals resulted in single and double breaks of the DNA strands. DNA double strand breaks (DSBs) are the most deleterious among various forms of distortion, because they involve both DNA strands and may result in the loss of genetic material. At large concentrations, oxygen-free radicals or more commonly reactive oxygen species (ROSs) may inflict harm to the composition of cells, lipids, proteins and DNA, resulting in oxidative stress that has been implicated in a variety of diseases.


1
3kV
DNA Leakage
Flow Rate Of O_2(sccm)
a.
b.
c.
d.
2
4kV
DNA Leakage
Flow Rate Of O_2(sccm)
a.
b.
c.
d.
3
5kV
DNA Leakage
Flow Rate Of O_2(sccm)
a.
b.
c.
d.
4
6kV
DNA Leakage
Flow Rate Of O_2(sccm)
a.
b.
c.
d.

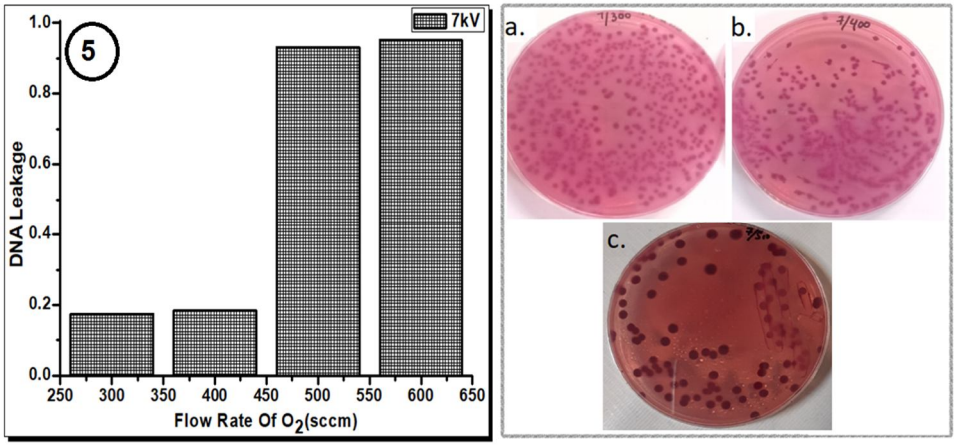


FIG. 5. Graphical visual representation of *E.coli* DNA distortion under different oxygen injection rates and applied potentials. (1). 3 kV applied potential (a) 300sccm (b) 400 sccm (c) 500sccm (d) 600 sccm. (2). 4 kV applied potential (a) 300sccm (b) 400 sccm (c) 500sccm (d) 600 sccm. (3). 5 kV applied potential (a) 300sccm (b) 400 sccm (c) 500sccm (d) 600 sccm. (4). 6 kV applied potential (a) 300sccm (b) 400 sccm (c) 500sccm (d) 600 sccm. (5). 7 kV applied potential (a) 300sccm (b) 400 sccm (c) 500sccm.

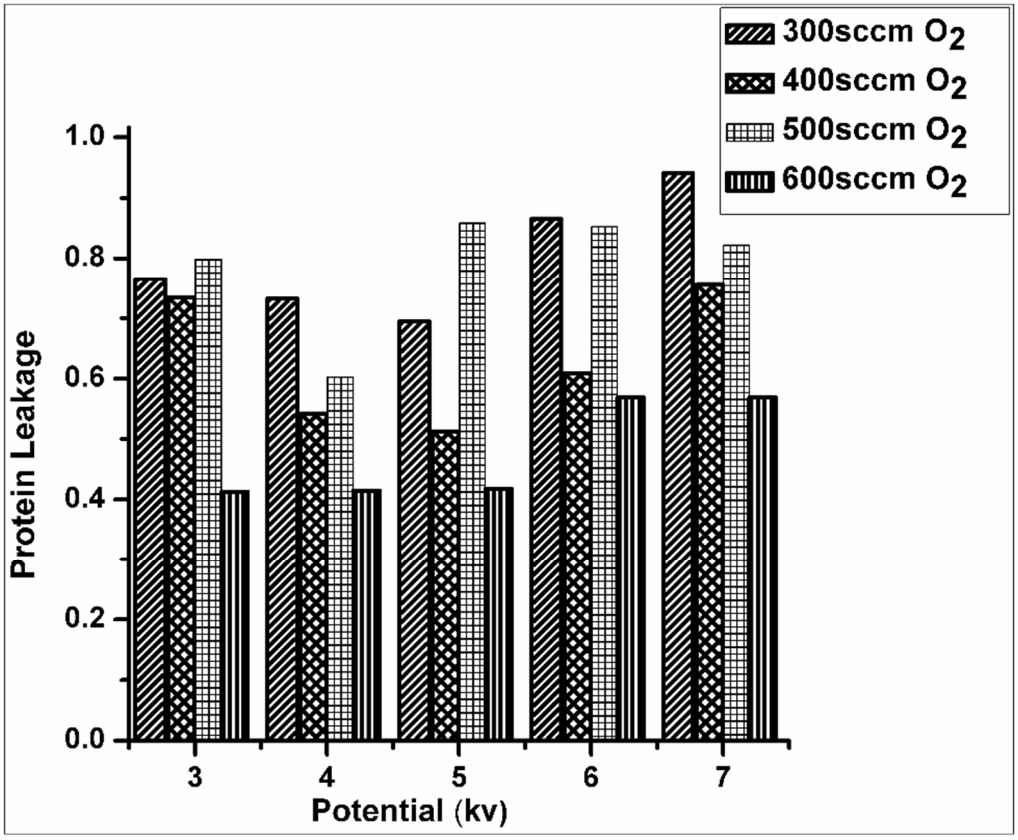


FIG. 6. Protein leakage test of *E.coli* under different oxygen injection rates and applied potentials.

4. Conclusions

Underwater plasma discharge is a novel technique for inactivation of micro-organisms. The inactivation of *E.coli* due to interaction of reactive oxidant species with DNA of gram negative *E.coli* has been studied, which reports that the injection rate of oxygen (300 to 600 sccm) and potential (3kV to 7kV) enhance the concentrations of $OH^{\cdot}$ radicals, O_3, reactive oxygen and UV radiations. At high oxygen injection rate and high potential, an increase in inactivation efficiency of *E.coli* is observed. The presence of ROSs resulted in DNA and protein leakage leads to the permanent inactivation of *E.coli*. Underwater plasma discharge is an effective and non-toxic approach for sterilization of water.

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