

## DBD Plasma Source for the Elimination of Naturally Occurring Microorganisms and Aerosolized Pathogens

### 3.1 Introduction

In the current era, infections transmitted through the air are causing diseases with significant morbidity and mortality [Leung, 2021]. Each year seems to bring a new influenza-like bacterium or virus that sparks an epidemic or pandemic [Piret et al., 2021]. Beyond human-to-human transmission, highly crowded and enclosed environments—such as healthcare facilities, schools, universities, large shopping malls, commercial buildings, and public spaces—facilitate the spread of indoor pathogens. These pathogens can be disseminated through heating, ventilation, and air conditioning (HVAC) systems, potentially leading to cross-infections. Indoor air quality (IAQ) is crucial for personal health and safety, often being 2 to 5 times, or even more, polluted than outdoor air [Van Tran et al., 2020]. Consequently, researchers have explored various methods to mitigate the risk of microbial infections in indoor spaces and enhance IAQ [Asilevi et al., 2021; Grinshpun et al., 2005; Guo et al., 2020; Roegiers et al., 2021; Van Durme et al., 2007; Wang et al., 2007].

One of the known techniques is chemical disinfection, which makes use of ethanol ( $C_2H_5OH$ ),  $H_2O_2$ , or a disinfectant [Cadnum et al., 2015]. Microorganisms can be eliminated as soon as they come into direct contact with these chemicals. However, it is challenging to decontaminate a big volume using this method. Another method of disinfection uses gases like ethylene oxide ( $C_2H_4O$ ) or  $O_3$  to prevent microbial infection [Moccia et al., 2020] [Muscarella, 2019]. With these methods, the sterilization is consistent across the treated volume, but it is essential to isolate the area and set up a suitable exhaust ventilation system. HEPA filters are also used for removing dust particles and airborne microorganisms from indoor environments [Lowther et al., 2020] [Chuaybamroong et al., 2010]. But HEPA filters do not deactivate microorganisms and are unable to filter small size aerosols and also these can cause pressure drops in air conditioning systems. Researchers have then moved on to UV and UV-based photocatalysis methods, which are quite promising for microbial reduction and indoor air purification [Li et al., 2021] [Mamaghani et al., 2017].

The UV-based approach to disinfection requires extended sterilization times [Lu et al., 2022] and struggles to be effective under conditions of either very low or very high pollutant concentrations [Liu et al., 2019]. Additionally, managing UV light in public spaces presents significant challenges [Ahlawat et al., 2022]. As a result, there is a pressing need for engineering control strategies that offer innovative solutions for the efficient disinfection of airborne pathogens. In this context, NTPs or cold plasma technologies have recently emerged as promising alternatives. These technologies have been extensively studied for their potential in sterilization and indoor air purification [Bahri et al., 2014; Ehlbeck et al., 2011; Pavlovich et al., 2018]. In line with this, a 1.5 log reduction (97%) in culturable *E. coli* was achieved by a DBD NTP reactor with a very high airflow rate (25 L/s) and a very short duration of plasma exposure [Gallagher et al., 2007]. The  $O_3$  concentration produced by the reactor was around 28 ppm, which is very high as per international health regulatory organizations' standards [Organización Mundial de la Salud (OMS), 2021]. A study carried by Nishikawa et al. [Nishikawa et al., 2001] has shown the effect of

negative and positive charged ions generated by DBD NTP plasma at ambient pressure on *Klebsiella coli*. They used an AC power source to produce discharge and achieved a maximum of 82% deactivation efficiency by operating the device for 60 minutes.

In this chapter, a cold-plasma device designed for environmental use is introduced, which simultaneously generates positive and negative ions along with  $\bullet\text{OH}$  to effectively eradicate both naturally occurring and aerosolized pathogenic microorganisms in enclosed environments [Jangra et al., 2023a]. The device operates on the principle of SDBD plasma, enhanced by a titanium dioxide ( $\text{TiO}_2$ ) nanoparticle catalyst, to achieve high-efficiency disinfection. The developed process for the production of positive and negative ions, and plasma generated  $\bullet\text{OH}$  is quite superior because these are produced by energetic electrons generated in the coaxial DBD geometry by cold plasma. In this study, the developed device generates  $\bullet\text{OH}$  and both positive and negative ions by harnessing high electrostatic fields with varying strengths, mimicking natural processes. Additionally, it sustains negative air ions for an average duration of over 25 seconds, which is particularly beneficial for deactivating long-lived pathogens. The device disinfection efficiency has been analyzed for TBCs and TFCs and also on aerosolized *E. coli* bacteria and MS2 phage in an indoor environment of  $3 \times 2.4 \times 2.4 \text{ m}^3$ . The obtained results are compared with the other indoor air treatment technologies for the effectiveness evaluation.

### 3.2 Experimental setup and Characterization

An already explained DBD plasma source in the section 2.2.1 has been used for the inactivation of naturally occurring microorganisms and aerosolized pathogens (*E. coli* and MS2 bacteriophage) from the enclosed environment of sizes  $28.3 \text{ m}^3$ . The plasma source comprises a coaxial DBD arrangement and an original/operational view of the source is shown in Figure 3.1. The external diameter of the dielectric tube is approximately 16 mm, while an aluminium electrode with a diameter of 12 mm is inserted into it, serving as the high-voltage electrode. This configuration creates a discharge gap/gap length of 2 mm. The plasma discharge is produced at atmospheric pressure, and ambient air is used as the gas carrier. A variable bipolar pulsed power supply (1-25 kV, 5-50 kHz PRF, 1 A, and 2  $\mu\text{sec}$  pulse width) is used to produce SDBD plasma. In this geometry, random local electric field generation is possible through the surface discharge, which is otherwise produced in volume dielectric discharge with much more complexities [Wang et al., 2018b].  $\text{TiO}_2$  NPs catalyst is coated on the outer grounded electrode via a dip-coating process. A uniform coating of thickness  $\sim 0.9\text{-}1.1 \mu\text{m}$  has been achieved in order to sustain electric fields of random strengths.

Firstly, the DBD plasma source has been characterized for its electrical and optical properties by means of V-I characteristics and OES. An air ion counter has been used to measure the concentration of negative and positive air ions generated from the DBD plasma source. The  $\bullet\text{OH}$  produced from the DBD plasma source are quantified using a widely used TA chemical probe approach. After the optimization of applied voltage, frequency and the parameters associated with them such as discharge power, concentration of negative and positive air ions and reactive species, the developed source has been further used for the elimination of microorganisms. For the assessment of naturally occurring microorganisms such as TBCs and TFCs, the plasma source has been tested in a room size measuring  $3 \times 2.4 \times 2.4 \text{ m}^3$  with and without ventilation. The plasma source is tested in a pre-cleaned closed test chamber ( $0.6 \times 0.6 \times 0.6 \text{ m}^3$ ) for the aerosolized pathogens. Various indoor air parameters have been also measured by using the different chemical probes. The complete experimental setup for the electrical and optical characterization as well as for the inactivation of microorganisms is similar as shown in Figure 2.5.



Figure 3.1: Original view of the coaxial DBD plasma device.

### 3.3 Experimental Observations and Analysis

#### 3.3.1 Electrical Characterization

A typical V-I characteristic of the above described coaxial DBD plasma source is shown in Figure 3.2. The electric power dissipated into the plasma is calculated from the waveforms of applied voltage (V) and the discharge current (I) using the equation 2.1 and 2.2, as described in section 2.3.1.

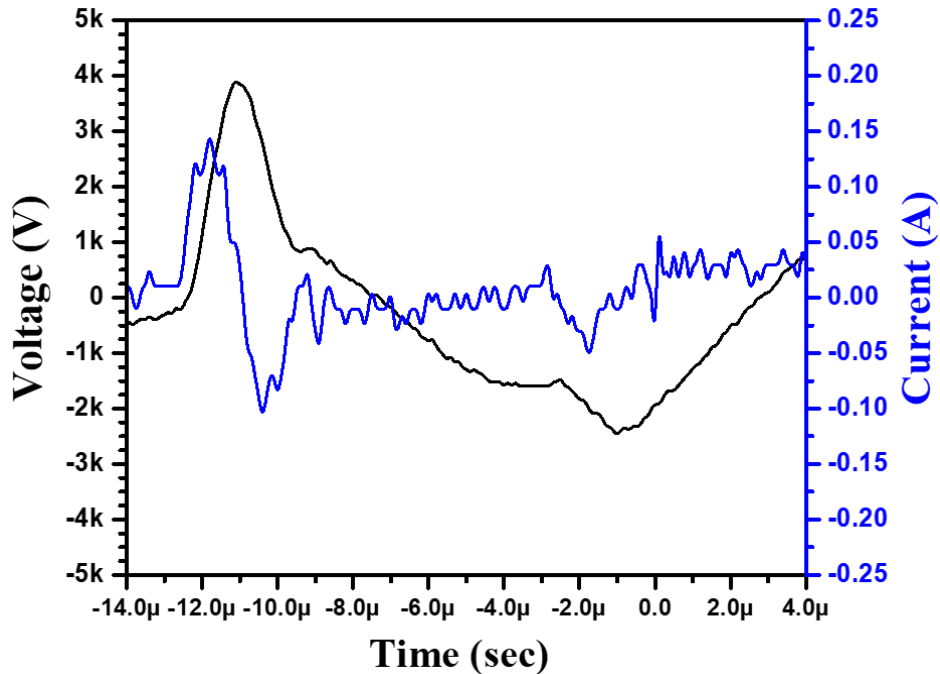


Figure 3.2: Typical V-I characteristics of the coaxial DBD source at 4 kV/20 kHz.

The energy consumed per pulse has been estimated by integrating power over the pulse duration. This gives a pulse energy of  $1.65 \times 10^{-4}$  Joule at the applied frequency of 20 kHz with an applied pulse voltage of 4 kV (peak). This results in 3.31 W average electrical power consumption in a coaxial DBD plasma device of length 100 mm. The power consumed per unit arc length by this device is  $\sim 0.33$  W/cm. From the V-I curve, the electron density is estimated using the equation 2.3. The electron density turns out to be  $\sim 1 \times 10^9$  particles/cm<sup>3</sup> at the aforesaid operational parameters.

### 3.3.2 Characterization of TiO<sub>2</sub> NPs Catalyst and its Coating on Electrode

The synthesized TiO<sub>2</sub> powder was characterized using the XRD system to determine the crystalline structure as shown in Figure 3.3. The lab-synthesized powder consists of TiO<sub>2</sub> NPs, both in the anatase and rutile phases, in which anatase is the predominant phase. The XRD pattern of the TiO<sub>2</sub> nanopowder (annealed at 450°C for 3 hours) revealed various strong diffraction peaks at  $2\theta$  values of 25.43° (101), 27.59° (101), 36.13° (103), 38.01° (004), 41.43° (111), 48.47° (200), 54.29° (105) 55.11° (211), 56.71° (220), 63.04° (24), 68.89° (110), 70.43° (220) and 75.31° (215) matching well with the TiO<sub>2</sub> anatase and rutile phase. The average crystalline size of the TiO<sub>2</sub> NPs is estimated by Debye-Scherrer's relationship (equation 2.7) [Bokuniaeva et al., 2019], and it is found to be  $\sim 18 \pm 2$  nm, confirming the nanoparticle formation of TiO<sub>2</sub>. These diffraction data are consistent with ICDD PDF 03-065-5714 for anatase TiO<sub>2</sub> and ICDD PDF 03-065-1118 for rutile TiO<sub>2</sub>.

For the morphological characteristics and to see the uniform distribution of prepared TiO<sub>2</sub> NPs, FESEM is used. Figure 3.4 shows the high magnification images of a synthesized TiO<sub>2</sub> powder sample. It can be noticed from the FESEM image that the average grain size of TiO<sub>2</sub> NPS is around  $22 \pm 2$  nm (as marked in yellow color), which is compatible with crystallite sizes estimated from the XRD data. The porosity network can also be observed from the FESEM image, which is better for releasing reactive ionic species from the interior nanoparticles as well.

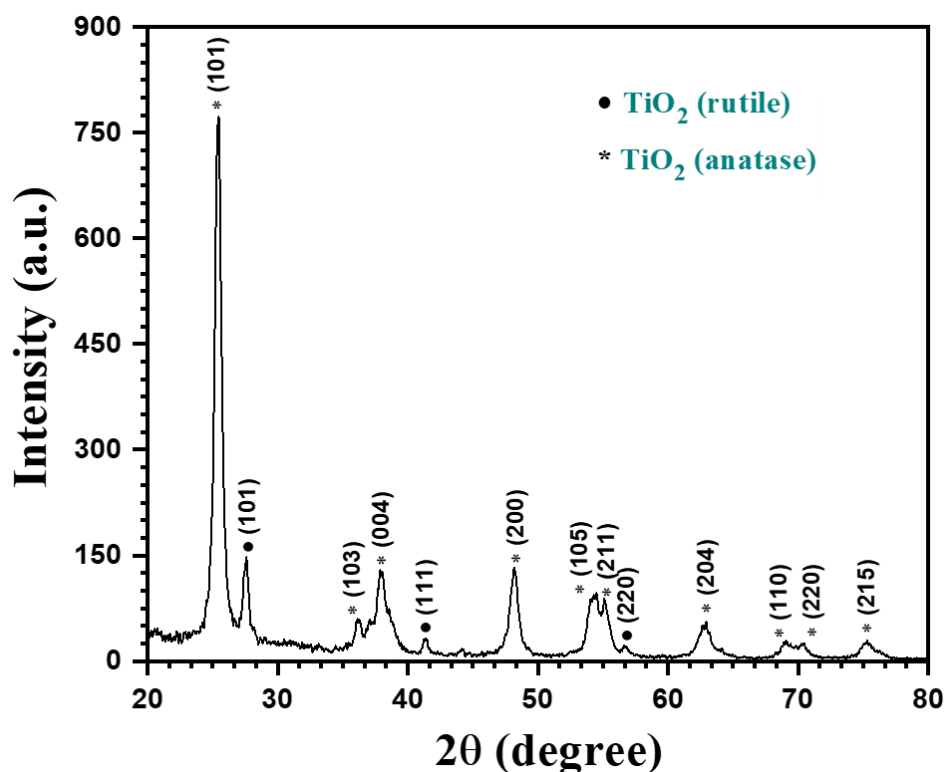


Figure 3.3: XRD pattern of synthesized TiO<sub>2</sub> powder annealed at 450°C for 3 hours.

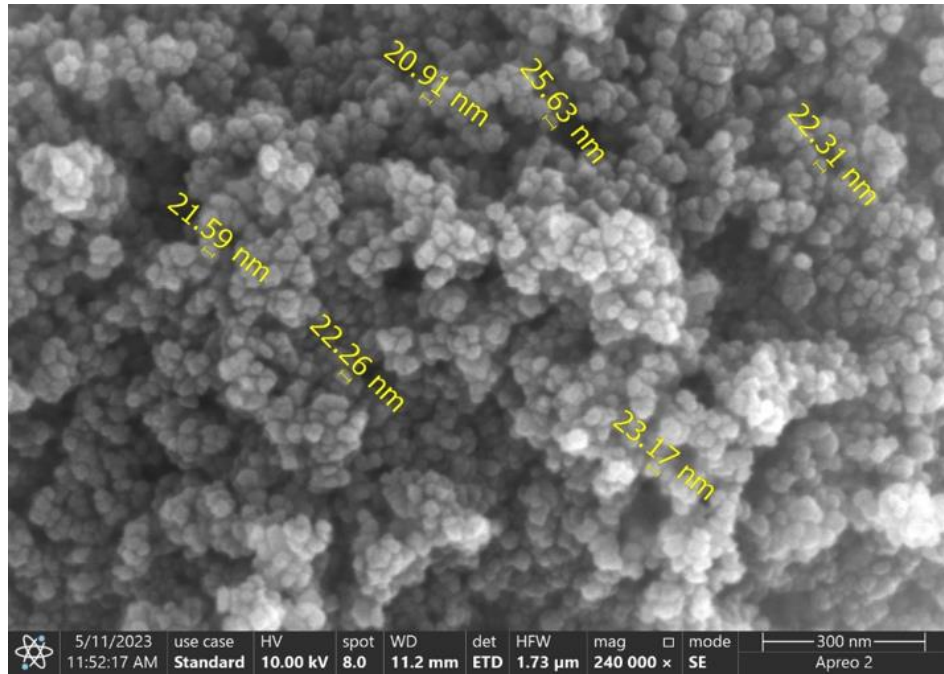


**Figure 3.4:** FESEM image of synthesized  $\text{TiO}_2$  powder annealed at  $450^\circ\text{C}$  for 3 hours.

A suspension of synthesized  $\text{TiO}_2$  NPs has been prepared for its dip coating on to the outer electrode of the plasma source. The SEM images together with respective energy dispersive x-ray (EDX) spectra of uncoated and  $\text{TiO}_2$  NPs coated outer electrodes are shown in Figure 3.5. The coating of  $\text{TiO}_2$  NPs on these structures is discernible [Figure 3.5 (d, e)] with respect to uncoated wire mesh electrode [Figure 3.5 (a, b)]. The EDX spectrum of uncoated wire mesh electrode structure shows the presence of elemental materials [Figure 3.5 (c)] and EDX spectrum of  $\text{TiO}_2$  NPs coated wire mesh electrode [Figure 3.5 (f)] shows the presence of titanium elemental peak together with a much larger fraction of oxygen on these structures.

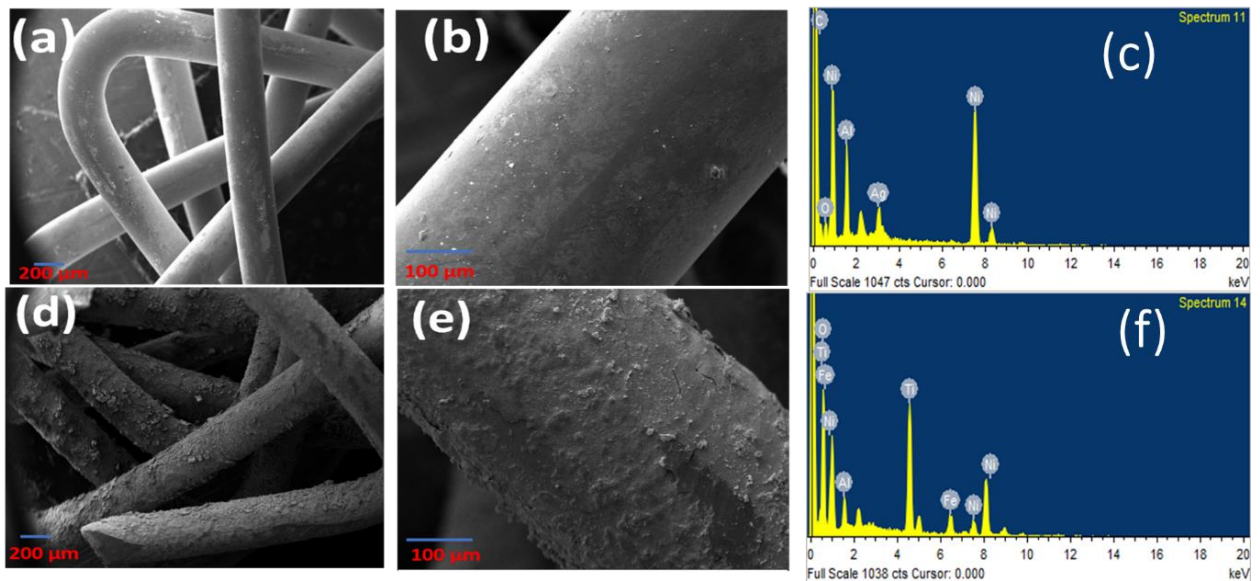


**Figure 3.5:** (a, b) SEM images, (c) EDX spectrum of uncoated wire mesh structure; and (d, e) SEM images, (f) EDX spectrum of  $\text{TiO}_2$  NPs coated wire mesh structures.

### 3.3.3 Active Air Ions and Hydroxyl Radicals

Figure 3.6 depicts the negative and positive air ion concentrations generated from the DBD plasma source at the outlet of the air purifying unit for different discharge powers in an enclosed environment. Positive and negative ions were generated by the molecules present in the air during their ionization by the discharge plasma produced at the contact points of the outer electrode. It is clearly seen that the densities of both negatively and positively charged ions enhance upon increasing the discharge power. The concentration of positive ions at the same discharge power is somewhat higher than the concentration of negative ions at ambient conditions. The measured value of negative and positive ions density is  $2.30 \times 10^5$  ions/cm<sup>3</sup> and  $2.42 \times 10^5$  ions/cm<sup>3</sup>, respectively at the discharge power 3.31 W. The concentrations of negative and positive ions produced from the device are such that they can yield local fields ( $> 10^6$  V/m) in the indoor environment similar to the bond energy of the chemical bonds of the harmful pathogens in the environment at their scales for faster deactivation.

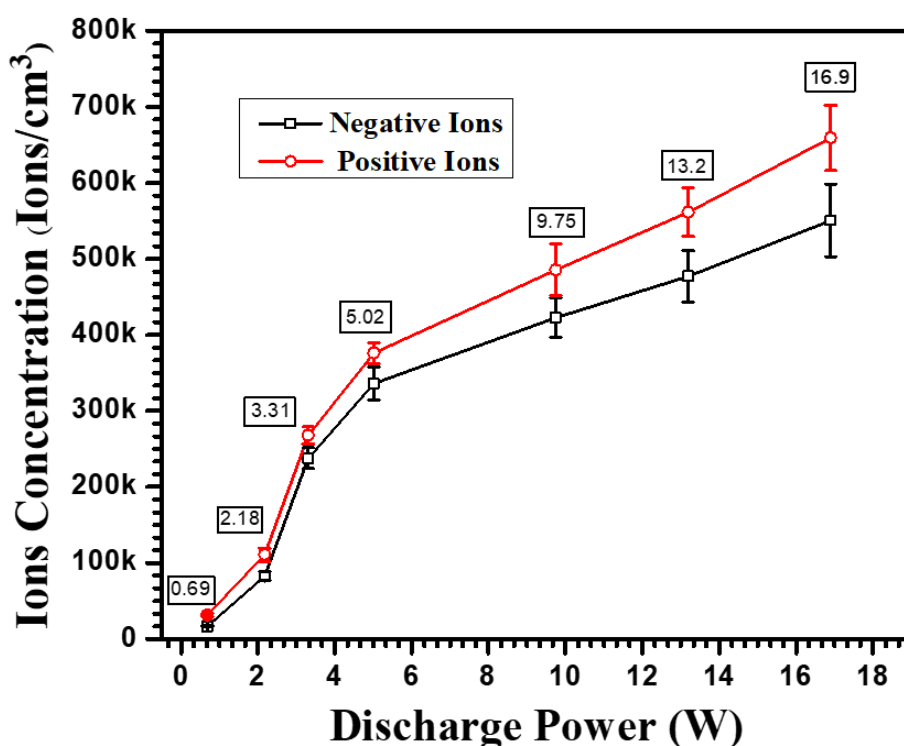


Figure 3.6: The concentration of negative and positive ions at different discharge powers.

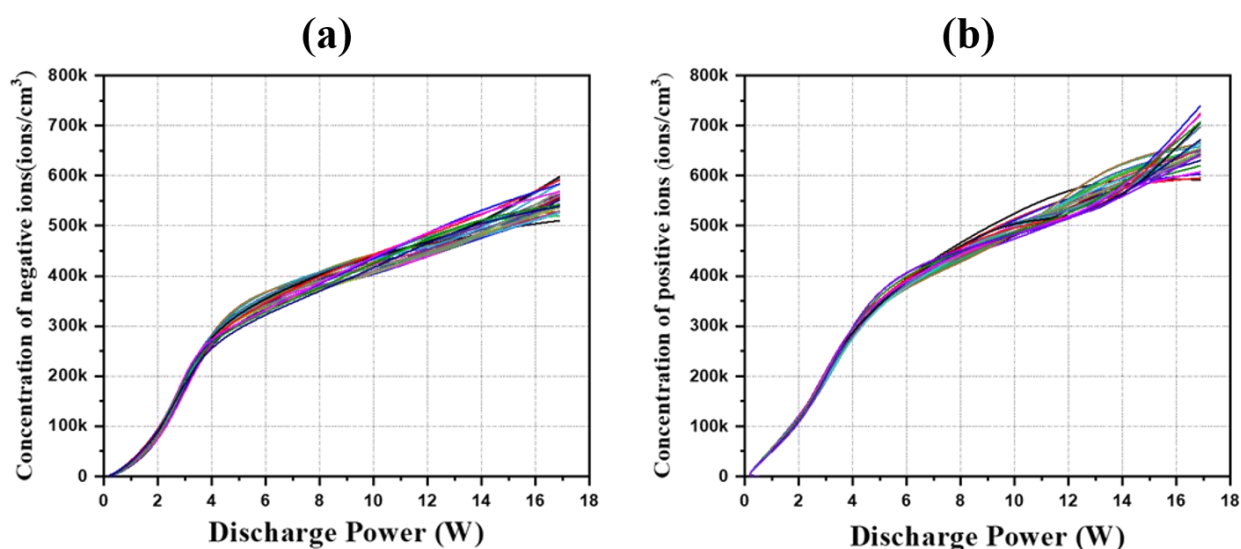


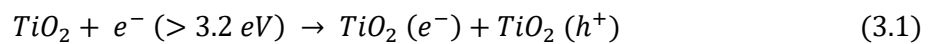
**Figure 3.7:** The concentrations of (a) negative and (b) positive air ions at different discharge powers at ambient conditions, monitored for 30 days.

We have also measured these positive and negative air ion concentrations for 30 days in the same indoor environment, and over that time the concentration approximately remained constant [see Figure 3.7 (a) and 3.7 (b)], indicating that there was no deterioration in the electrodes and TiO<sub>2</sub> coating. Furthermore, the local fields are such that the excess negative and positive ion concentrations are neutralized in air. The negative ions obtained are a mix of hydroxyl ions produced by activating the TiO<sub>2</sub> catalyst in the surface DBD.

It should be noted that, in general, a very basic "Corona" equilibrium model is utilized to comprehend the discharge plasma spectroscopically when electron density is low [Hutchinson, 2002]. In the corona model, it happens that all upward transitions are collisional and all downward transitions are radiative. In fact, in a low-density plasma case ( $n_e \leq 10^{10} \text{ cm}^{-3}$ ), the probability for spontaneous decay of an excited atom is much higher than that of any collisional depopulation process. Hence it can be easily assumed that,

- (i) All upward transitions are collisional
- (ii) All downward transitions are radiative

This can only happen when transitions are mostly occurring in the excited states but near to the ground state, which implies that the spectrum at low electron densities mostly lies in the visible region [Fantz, 2006]. Further, these assumptions are also valid even for  $n_e \leq 10^{10} \text{ cm}^{-3}$  but the transitions need to happen very near to the ground state. Such transition produces spectrum in the extreme ultraviolet (EUV) and/or vacuum ultraviolet (VUV) region of the spectrum, which is in one proof that there is almost nil UV generation during the discharge. A test done with a calibrated UV-C radiometer has shown negligible UV generation during the discharge. Assadi et al. [Assadi et al., 2014] reported that the UV light generated via a surface DBD plasma was too weak to activate the TiO<sub>2</sub> catalyst. Wallis et al. [Wallis et al., 2007] suggested that the impact of plasma-generated electrons on the surface of TiO<sub>2</sub> can produce  $e^- - h^+$  pairs similar to photon absorption since the energy of these electrons, i.e., 3 - 4 eV is identical to the photons.

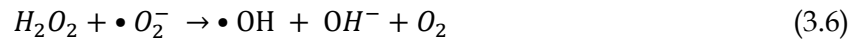
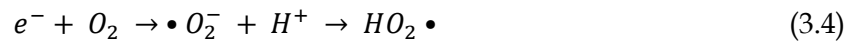


The recombination time of these  $e^- - h^+$  pairs is 2-3 times faster than the charge separation time. Since we are able to generate electric fields of random strengths, the recombination time of these  $e^- - h^+$  pairs would have reduced. This is well supported by Mizuno et al. [Mizuno et al., 1999], they explained that the electric fields produced by the interactions of discharge plasma and metal oxide photocatalyst significantly shorten the time required for recombining  $e^- - h^+$  pairs. Probably electrons and holes produced by the HEEs would have been transported in the opposite direction due to the action of these randomly created electric fields and that can minimize the probability of their recombination. This can obviously lead to increased negative ion sustenance time.

Figure 3.8 (a) shows the concentrations of the measured negative air ions and their sustenance time for different discharge operating parameters. We measured the negative ions concentration produced by the device at 30 cm from the plasma source. The used AIC sucks the air at a calibrated rate and, in general, the display shows the ion count after 2 seconds that is continuously displayed. By using an analog output jack, the long-term automated monitoring of ion counts, i.e., up to 30 seconds is possible. Accordingly, the measured ion densities at different operating parameters have been traced for more than 25 seconds. Since the average ion densities

measured during this time scale is much higher than the original room ion concentration which was  $\sim 200 - 300$  particles/cm<sup>3</sup> in the closed test environment chosen in the study, it clearly shows that the average sustenance time of ions is more than 25 seconds. Accordingly, the average measurement of these ions has been used to show the concentrations of the plasma detergent ions in the environment. Figure 3.8 (b) shows the average negative ion concentration at 15 cm and 170 cm from the plasma source. Given the fact that the air purifying unit is positioned in the middle of the room, having a length of around 300 cm, it is necessary to measure the ion concentration at a minimum distance of 150 cm, in order to evaluate the impact of reactive species and active air ions on the inactivation of naturally occurring microorganisms throughout the enclosed space. The concentration of negative air ions at a distance of 15 cm is around  $2.30 \times 10^5$  ions/cm<sup>3</sup>, which decreased to 8,000 ions/cm<sup>3</sup> at a distance of 170 cm away from the plasma source. The values of negative ion concentrations decreased with distance due to their expansion in the enclosed environment. Still, the average sustenance time of negative ions is the same.

The generated electron-hole pairs, negative and positive air ions can lead to generate cold-plasma detergent ( $\bullet\text{OH}$  and  $\text{OH}^-$  negative ions) in the environment by the following reactions;



For the quantification of  $\bullet\text{OH}$ , we have utilized a TA probe, as described earlier in section 2.3.5. Figure 3.9 (a) shows the fluorescence spectra of aqueous TA solutions exposed to the air purifying unit to confirm that the developed device is generating  $\bullet\text{OH}$  in the environment. The generation of the  $\bullet\text{OH}$  has been analyzed for different exposure times, i.e., 5 minutes, 10 minutes, 15 minutes, 20 minutes, 25 minutes, and 30 minutes. Samples of  $\sim 5$  mL treated TA solutions were analyzed within three hours of performing the experiments through a fluorescence spectrophotometer. The excitation wavelength was set at 315 nm, and the fluorescence spectra were collected in the spectral range between 350 nm to 550 nm at an emission wavelength of 425 nm. The TA can be oxidized into HTA by  $\bullet\text{OH}$  in an aqueous solution, which emits light at 425 nm [(see Figure 3.9 (a))]. The peak for these samples has shown consistent enhancement in the intensity at the emission wavelength that confirms the continuous generation of  $\bullet\text{OH}$  from the device.

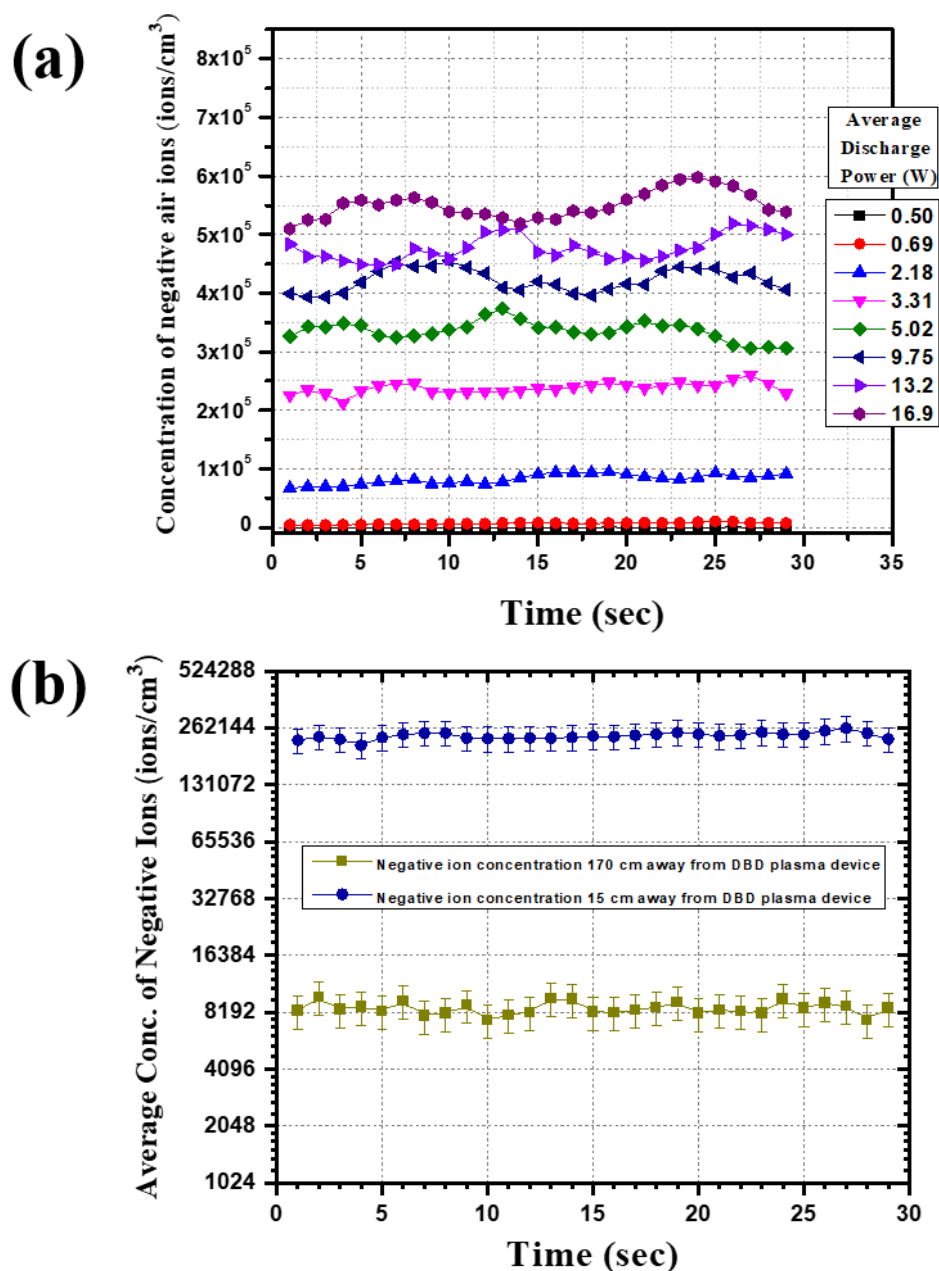


**Figure 3.8:** The recorded average concentrations of negative air ions with their sustenance time (a) for different average discharge powers; (b) for fixed distances from the device at 15 cm and 170 cm.

Figure 3.9 (b) shows the time-dependent variation in the concentration of  $\bullet\text{OH}$  in the HTA solution. By using a calibration curve of HTA (see Figure 2.10) the exact concentration of  $\bullet\text{OH}$  in the HTA can be obtained. As the exposure time progressed, the concentration of  $\bullet\text{OH}$  is also increased in the HTA solution. When the treatment time varied from 5 minutes to 30 minutes, the concentration of  $\bullet\text{OH}$  significantly increased, almost linearly, from 22.78  $\mu\text{M}$  to 87.24  $\mu\text{M}$ . Within the 30 minutes of timescale, the rate of formation of  $\bullet\text{OH}$  was found to be  $1.74 \times 10^{-8} \text{ M/s}$ .

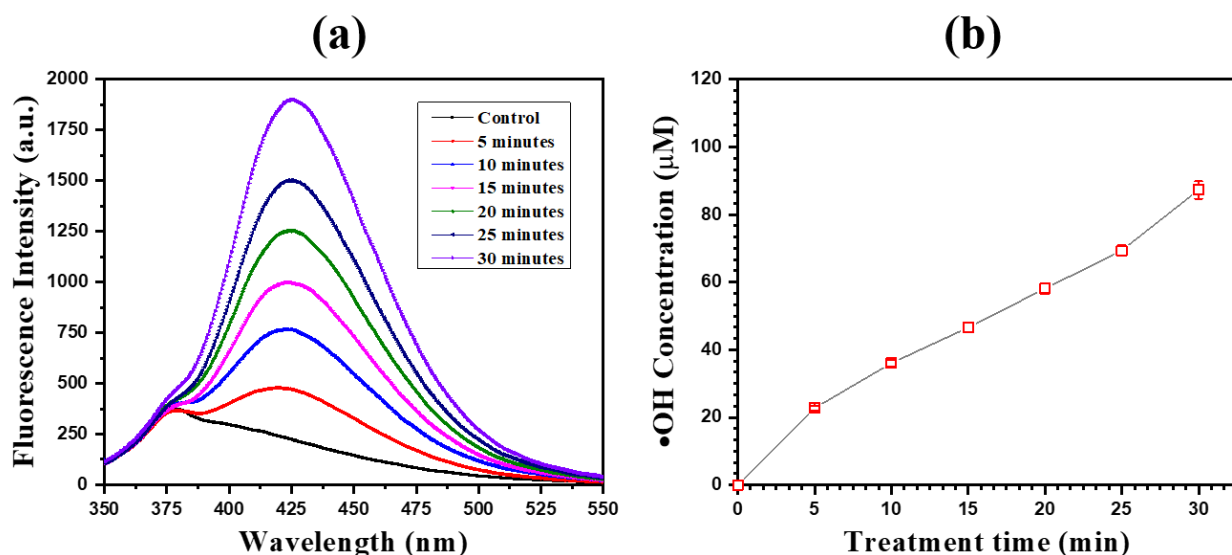


**Figure 3.9:** (a) The fluorescence spectra of the HTA solutions and, (b) the time-varying concentrations of •OH generated by the developed device at 4 kV/20 kHz.

### 3.3.4 Assessment of Various Indoor Air Parameters

While the production of •OH is safe, the emission of other indoor air parameters such as benzene, benzo pyrene, O<sub>3</sub>, NO<sub>2</sub>, CO, and others must be closely monitored and adhere to all international health regulatory organizations standards. Because we coexisted with •OH during evolution, neither they can penetrate our bodies nor those of animals, insects, or plants. The body's cells have created a variety of enzyme and non-enzyme-based antioxidant systems to neutralize free radicals. In particular, the skin and mucous membranes have developed into barriers that can neutralize radicals, preventing damage to human structures. We have investigated ten indoor air parameters – NO<sub>2</sub>, SO<sub>2</sub>, CO, O<sub>3</sub>, NH<sub>3</sub>, benzene, benzo pyrene, arsenic, lead, and nickel in an enclosed environment. Most of them such as SO<sub>2</sub>, benzene, benzo pyrene, arsenic, lead, and nickel, are observed below the limit of quantification (BLQ) in just 15 minutes of continuous operation of the device, while NH<sub>3</sub> concentration reached BLQ in just 30 minutes of continuous operation (see Table 3.1). O<sub>3</sub> and NO<sub>2</sub> are the main byproducts of atmospheric DBD, which are formed due to the following reactions:



O<sub>3</sub> is formed by the three-body collision reaction between atomic oxygen and O<sub>2</sub>, here, M can be molecular oxygen/nitrogen [Atkinson et al., 2006]. However, in this study, the concentration of NO<sub>2</sub> is first found to increase till 900 mg/m<sup>3</sup> (starting from 425 mg/m<sup>3</sup>) up to 45 minutes of continuous exposure. After that, it attains saturation [see Figure 3.10 (a)], which indicates no formation of excessive NO<sub>2</sub> by the developed plasma source.

The O<sub>3</sub> concentration has also been measured around the air purifying unit using a portable O<sub>3</sub> gas detector, which is found to be 129 ppb at the aforementioned operational parameters, which is within the permissible exposure limit as per the Centre for Disease Control (CDC) guidelines [US Department of Labor, 1976]. Concentration of O<sub>3</sub> produced by the DBD plasma source at different discharge powers is shown in Figure 3.10 (b).

**Table 3.1:** Summary data of different indoor air parameters.

| Time (min) | Sulphur Dioxide | Ammonia | Carbon Monoxide | Benzene | Benzo Pyrene |
|------------|-----------------|---------|-----------------|---------|--------------|
| 0          | 4.98            | 178     | 6.4             | BLQ     | BLQ          |
| 15         | BLQ             | 77      | 6.5             | BLQ     | BLQ          |
| 30         | BLQ             | BLQ     | 6.4             | BLQ     | BLQ          |
| 45         | BLQ             | BLQ     | 6.5             | BLQ     | BLQ          |
| 60         | BLQ             | BLQ     | 6.5             | BLQ     | BLQ          |
| 90         | BLQ             | BLQ     | 6.5             | BLQ     | BLQ          |
| 120        | BLQ             | BLQ     | 6.5             | BLQ     | BLQ          |

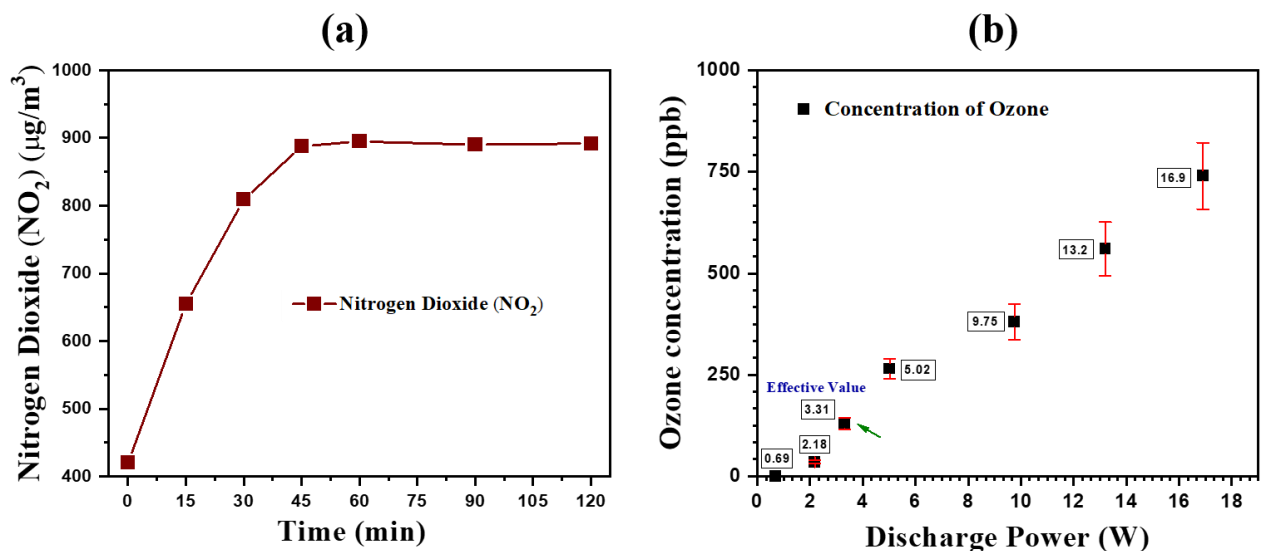


**Figure 3.10:** (a) NO<sub>2</sub> concentration during 120 minutes of continuous operation of DBD plasma source at an optimized parameters of 4.0 kV/20 kHz, (b) Concentration of O<sub>3</sub> produced by DBD plasma source at different discharge powers.

### 3.4 Inactivation of Naturally Occurring and Aerosolized Pathogens

To investigate the elimination of TBCs and TFCs by the developed cold-plasma detergent in environment device, the air purifying unit was operated at the optimized parameters (4 kV/20 kHz) in an indoor space measuring 3 × 2.4 × 2.4 m<sup>3</sup> with and without ventilation. Air sampling was carried out with the air sampler before operating the device and then after 15 minutes, 30 minutes, 45 minutes, 60 minutes, 90 minutes, and 120 minutes of continuous operation. TBCs concentrations in the test room's indoor environment were 175 CFU/m<sup>3</sup> and 295 CFU/m<sup>3</sup>, for with and without ventilation case, respectively. Figure 3.11 (a) illustrates the deactivation efficiency of TBCs at different time intervals in both cases. In the case of without ventilation, more than 99% deactivation efficiency has been achieved in 90 minutes of continuous operation of the plasma source, while more than 94% deactivation efficiency has been achieved in 60 minutes of continuous operation of the source. In the case of with ventilation, the deactivation efficiency dropped slightly. Around 92% deactivation efficiency has been achieved in around 120 minutes.

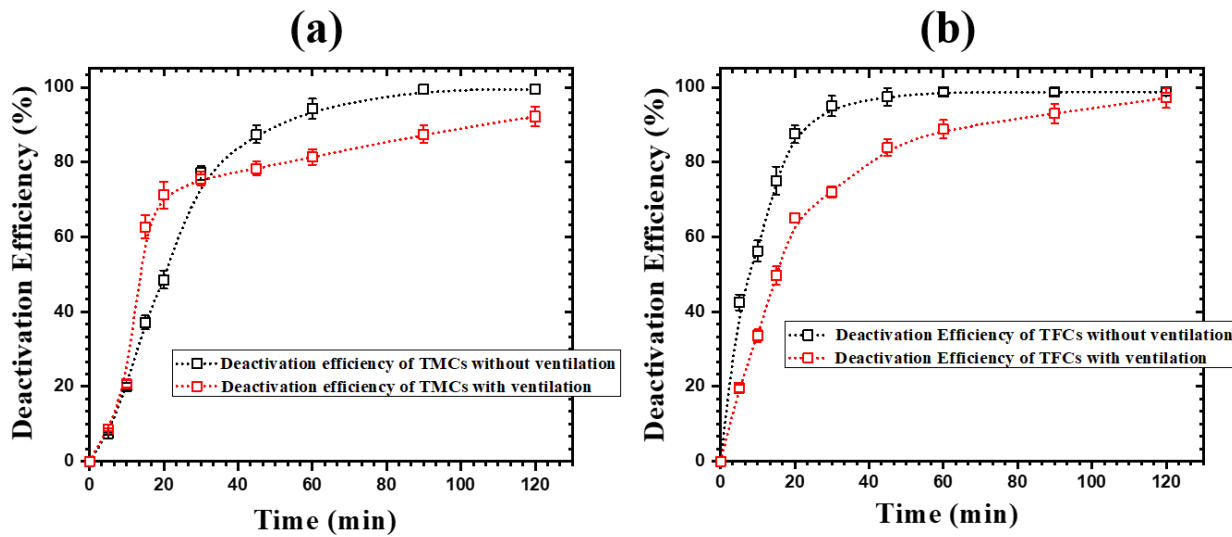


**Figure 3.11:** The deactivation efficiencies of (a) total bacterial counts (TBCs) and (b) total fungal counts (TFCs) in with and without ventilation cases.

Figure 3.11 (b) shows the deactivation efficiency of TFCs at different time intervals in both cases. The controls of TFCs were around 80 CFU/m<sup>3</sup> and 143 CFU/m<sup>3</sup>, respectively, for with and without ventilation cases. In continuous 30 minutes of operation of the plasma source, more than 95% deactivation efficiency has been achieved, and a maximum of more than 99% deactivation efficiency has been achieved in 60 minutes of continuous operation for the case of without ventilation. On the other hand, with ventilation, the deactivation efficiency has been around 72% and 89% in continuous 30 minutes and 60 minutes of operation of the source, respectively. Tests have also been performed to see the natural decay of the counts of TBCs and TFCs in both the cases, with and without ventilation. The comparative results of their decay along with natural decay are shown in Figure 3.12 and no significant effect of natural decay has been observed in the tested environment. A similar study in a closed chamber was carried out by Comini et al. [Comini et al., 2021] and showed that by positive and negative air ions, more than 70% inactivation of *E. coli* took about 8 hours.

Results suggest that the developed plasma source is producing positively and negatively charged ions along with •OH which are utilized to eliminate different airborne and pathogenic microorganisms. The lipid peroxidation can produce transient pores through cold-plasma detergent ions (or reactive species) for wall rupture of bacteria/viruses in the indoor environment. Furthermore, since the plasma discharge happens in the air, in balancing positive and negative ions, the electron pulsation will receive a certain amount of energy. When the electrons collide with the bacteria and the mold scorpion, the energy transmitted will be similar to the bond energy of the chemical bond, the bond will be broken and bacteria/mold/fungus may no longer breed [Pillet et al., 2016].

The plasma source efficiency in the reduction of aerosolized *E. coli* bacteria and MS2 bacteriophage in an enclosed environment measuring size 0.6 x 0.6 x 0.6 m<sup>3</sup> was also tested. These aerosolized microorganisms were selected specifically for their ability to gauge the efficacy of the developed source for reducing a commonly encountered organism, such as the influenza virus and SARS-CoV-2. The net log reduction and net percentage reduction data are shown in Figure 3.13 and Table 3.2, respectively.

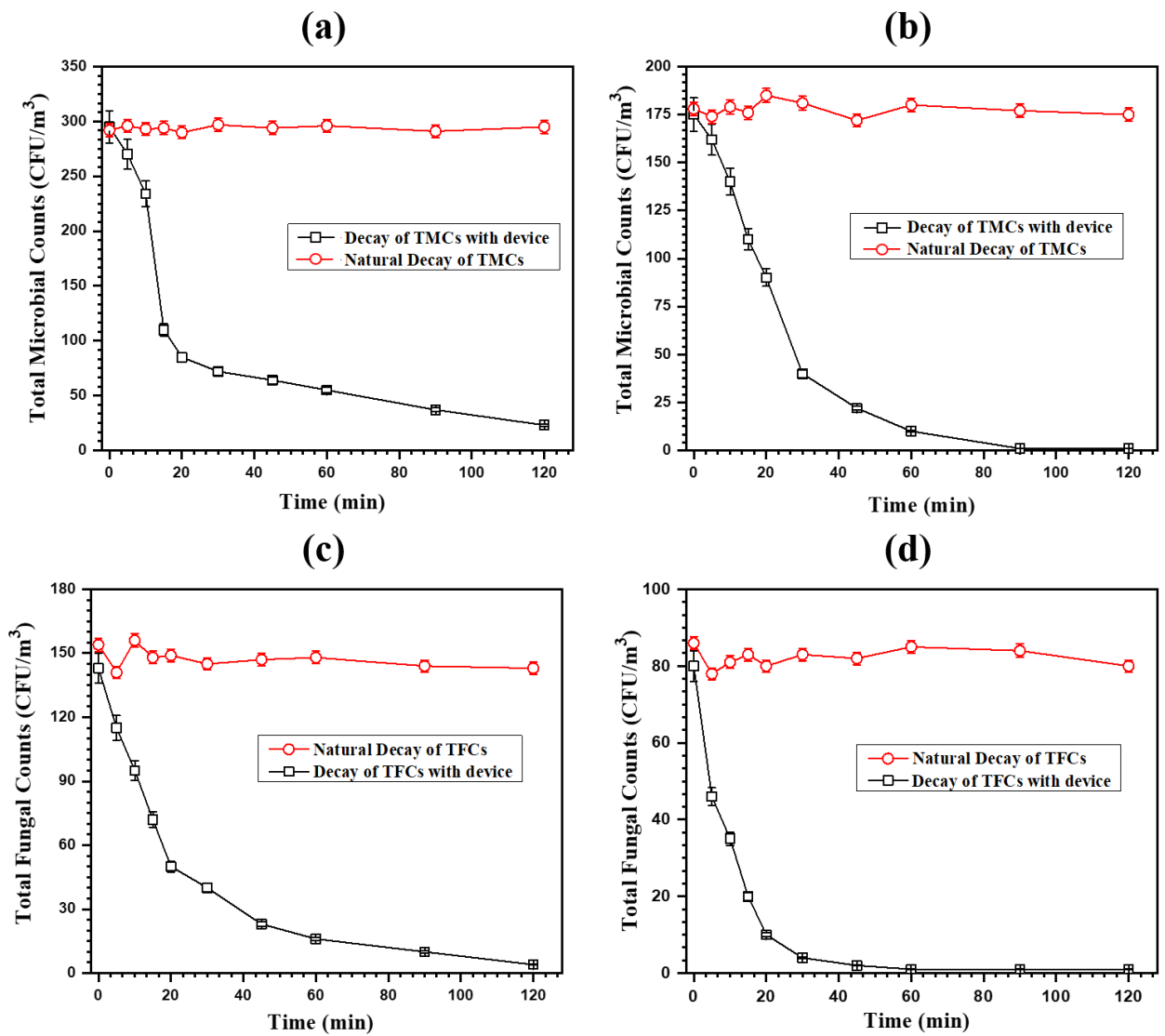


**Figure 3.12:** Decay of the counts of TBCs and TFCs naturally and with the operation of cold plasma detergent device (a, c) in the case of ventilation, (b, d) in the case of without ventilation.

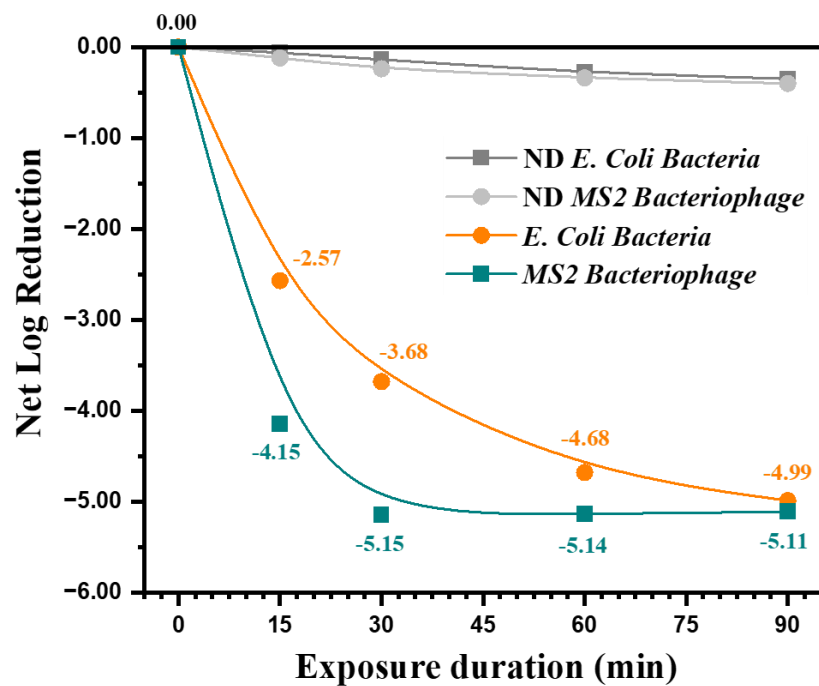


**Figure 3.13:** Net log reduction in case of aerosolized MS2 phage and *E. coli* bacteria.

**Table 3.2:** Deactivation effectiveness of aerosolized MS2 phage and *E. coli*.

| Bioaerosol type | Species        | Data type             | 15 minutes | 30 minutes | 60 minutes | 90 minutes |
|-----------------|----------------|-----------------------|------------|------------|------------|------------|
| <b>Virus</b>    | MS2 phage      | Initial Log (Control) | 5.14±0.25  | 5.14±0.26  | 5.13±0.24  | 5.11±0.24  |
|                 |                | Net Log Reduction     | 4.15±0.21  | 5.14±0.26  | 5.13±0.26  | 5.11±0.25  |
|                 |                | Net % Reduction       | 99.993     | 99.999     | 99.999     | 99.999     |
| <b>Bacteria</b> | <i>E. coli</i> | Initial Log (Control) | 4.98±0.26  | 4.98±0.24  | 4.97±0.25  | 4.99±0.25  |
|                 |                | Net Log Reduction     | 2.57±0.13  | 3.68±0.18  | 4.68±0.23  | 4.99±0.25  |
|                 |                | Net % Reduction       | 99.733     | 99.979     | 99.998     | 99.999     |

When tested against MS2 bacteriophage, the device showed a maximum of 99.999% reduction in 30 minutes while reaching a 99.99% reduction, it took only 15 minutes. When tested against *E. coli*, the developed device has shown a maximum reduction of 99.999% in 90 minutes while reaching a 99.98% reduction, it took 30 minutes. In both cases, the initial reduction is very fast, more than 99% in 15 minutes of continuation operation of the air purifying unit. In this study, the phenomenal performance of the airborne microorganism's elimination has been accomplished through the combination of negative and positive ions with the •OH.

The decrease in the number of CFUs and PFUs is mostly caused by the cold-plasma detergent as the exposure time increases. Especially for the *E. coli* bacteria, the reduction in CFUs is comparatively lower with increasing treatment time because of their self-defense and self-healing mechanisms that protect them [Vijay et al., 2013] [Song et al., 2019]. In contrast, when the plasma exposure time is longer, a higher number of cold-plasma detergent species are generated, causing damage to the outer cell membrane of *E. coli* bacteria, which immediately suppresses the self-healing protection mechanism of the *E. coli* bacteria, resulting in a higher deactivation efficiency. In general, there are multiple repair systems in bacteria, i.e., *E. coli*, such as lipid remodeling (one of the outer membrane components), membrane repair proteins such as MreB and MreD, and enzymes such as catalase, superoxide dismutase (neutralize the ROS), and autolysins. Besides these, there is a stress response that can activate the gene series. These genes help activate sigma factors such as sigmaB and sigmaE. All these factors repairs the damaged cell wall. Self-defence in *E. coli* includes bacteriocins (kills other bacteria), efflux pump (expel antibiotics out from the cell), sigma factor RpoS (activate during stress), quorum sensing, and genetic changes such as gene transfer (for acquiring the antibiotic resistance) [Parsons et al., 2013] [Nikaido et al., 2012].

Table 3.3 shows a comparison of the log reduction of MS2 bacteriophage with different NTP reactor configurations known in the literature. As quite evident, the log reduction of MS2 bacteriophage in this study is higher than that of the earlier known works using different NTP configurations. The time taken by the system to achieve maximum log reduction is also lower than that using different NTP reactor configurations. In fact, the complete deactivation of any virus depends on different properties, like its genome type (i.e., ssRNA or dsRNA), enveloped or

non-enveloped, and its genome size. If we compare the different characteristics of SARS-CoV-2 and MS2 bacteriophage, then the genome size of SARS-CoV-2 is 29.8 kb (enveloped), while the genome size of MS2 bacteriophage is 3.6 kb (non-enveloped). Viruses with larger genomes generally inactivate more quickly because their larger size makes them more susceptible to hydroxyl radical-based damage. Due to its lower genome size and non-enveloped characteristics, it is a bit difficult to deactivate MS2 bacteriophage in comparison to the SARS-CoV-2 [Blatchley et al., 2021].

**Table 3.3.** A comparison of log reduction of MS2 bacteriophage with different NTP reactor configurations.

| Sr. No. | Virus                        | NTP Reactor Configuration    | Initial Concentration   | Log Reduction | Time (Minutes) | References             |
|---------|------------------------------|------------------------------|-------------------------|---------------|----------------|------------------------|
| 1       | MS-2 bacteriophage           | Packed-Bed Reactor           | $10^6$ pfu/ml           | 2.35          | 45             | [Xia et al., 2019b]    |
| 2       | MS-2 bacteriophage (on FFRs) | SDBD + Water Mist            | $10^6 - 10^8$ pfu/piece | 2.00          | 20             | [He et al., 2022]      |
| 3       | MS-2 bacteriophage           | Corona Discharge             | $10^{10}$ pfu/ml        | 4.00          | NA             | [Whiley et al., 2022]  |
| 4       | MS-2 bacteriophage           | PCO + HEPA Filter            | $10^{10}$ pfu/ml        | 4.00          | 35             | [McLeod et al., 2021]  |
| 5       | MS-2 bacteriophage           | Bi-Polar Ionization & PCO    | $10^5$ pfu/ml           | 0.88 & 1.80   | 60             | [Ratliff et al., 2022] |
| 6       | MS-2 bacteriophage           | SDBD (Cold-Plasma Detergent) | $> 10^5$ pfu/ml         | $> 5.00$      | 30             | This Work              |

The above comparison shows the effectiveness of the developed device in the elimination of aerosolized pathogens. The scope of this device is not limited only in the elimination of TBCs, TFCs, but it can also deactivate the most lethal range of other airborne bacteria and viruses perhaps including SARS-CoV-2, SARS CoV, Influenza, etc. It is because the virus is not a living organism, but a protein molecule covered by a protective layer of lipid (fat) and the produced active plasma detergent ions for more than 25 seconds with optimum concentration can deactivate such viruses. It is a well-known fact that any soap or detergent is the best remedy for Influenza viruses from hands because the foam cuts the fat. One needs to rub so much, for ~ 20 seconds or more, to make a lot of foam. By dissolving the fat layer, the protein molecule disperses and breaks on its own. It is expected that the generated cold plasma detergent with sufficient average sustenance time of active negative ions in the environment can work well for the long living pathogens, such as SARS-CoV-2, SARS-CoV, Influenza, etc.

### 3.5 Conclusion

In summary, we have developed a portable cold-plasma detergent in environment device for efficient generation of active negative and positive air ions along with  $\bullet\text{OH}$  similar to Mother Nature in a coaxial DBD configuration. The device is able to generate efficient plasma detergent

ions in the indoor environment without any additional gas and works at atmospheric pressure. A 100 mm device is able to produce active ions with concentrations varying from 300 ions/cm<sup>3</sup> to 5,00,000 ions/cm<sup>3</sup> and above. It is also able to generate negative ions with an average sustenance time of more than 25 seconds. The device is easily scalable in length, width and ion concentrations. The average consumed power per unit arc length by this device is found to be ~ 0.33 W/cm. The device is quite effective against aerosolized pathogens and is capable of reduction of TBCs and TFCs by more than 99%, *E. coli* bacteria by more than 99.999% and RNA-based virus -MS2 bacteriophage by more than 99.999% in a shorter operation time (< 90 mins) of the air purifying unit in the indoor air environments tested in sizes up to 3 x 2.4 x 2.4 m<sup>3</sup>. The synergistic effect of both air ions along with the generated •OH are possibly accountable for the obtained higher disinfection efficiency.

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