

The Role of Active Air Ions and Hydroxyl Radicals on the Eradication of ESKAPE Bacteria using Cold Plasma

6.1 Introduction

The ESKAPE bacteria, which includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*, are recognised as pathogens commonly linked to hospital-acquired infections (HAIs). They present different challenges in terms of disease development, spread, and resistance, posing a significant risk to public health. [Rice, 2008] These microorganisms are the primary cause of challenging HAIs because of their capability to bypass the antimicrobial action of antibiotics [Rice, 2008]. According to estimates from the WHO, each year, HAIs harm hundreds of millions of people globally, resulting in billions of dollars spent on associated expenditures [O'Connor et al., 2014] [Haque et al., 2018]. Infections from ESKAPE pathogens lead to significant morbidity and mortality rates, prolonged hospitalisations, and increased treatment expenses [Friedman et al., 2016]. Different techniques have been utilised to eliminate ESKAPE pathogens, including chemical disinfection [Chen et al., 2020], gaseous disinfection [Ibáñez-Cervantes et al., 2023] [Westover et al., 2023], and UV irradiation [Ahlawat et al., 2023] [Rangel et al., 2022]. However, decontaminating the entire area using these methods is challenging due to their numerous drawbacks, as reported elsewhere [Song et al., 2022]. For example, in the case of chemical or gaseous disinfection, bacteria can be eradicated by direct exposure to the disinfectant. Therefore, it is necessary to establish proper ventilation for gas exhaust, and the area must be isolated. Novel techniques for infection prevention and treatment are required to address the increasing challenge posed by multidrug-resistant bacteria and nosocomial infections. NTP or cold plasma is an emerging promising solution for treatment and disinfection that does not require any isolation or any specific arrangements [Das et al., 2023b; Modic et al., 2017; Scholtz et al., 2021].

NTPs have received significant interest as a potential technique for controlling microbiological organisms [Das et al., 2023a; Gupta et al., 2019; Liang et al., 2012]. Sarthak et al. [Das et al., 2023a] studied the impact of direct treatment of a cold atmospheric plasma jet on ESKAPE bacteria and observed that oxidative stress caused by RONS and electrostatic stress from air ions are key factors in eradicating the bacteria. Flynn et al. [Flynn et al., 2015a] investigated the effect of atmospheric pressure non-thermal plasma jet on ESKAPE pathogens in the biofilm mode and found that ESKAPE bacteria were eliminated after only 360 seconds of direct plasma (when plasma is in contact with the target pollutant) treatment.

There have been few investigations on the use of NTPs for the indirect treatment of ESKAPE infections, which occurs when plasma does not come into contact with the target pathogens. Also, there is a lack of comprehensive studies regarding the influence of bipolar pulsed-powered DBD plasma on eliminating ESKAPE pathogens. Bipolar pulse-powered DBD plasma has many advantages over AC, DC, RF, and microwave due to delivering energy in short and high-voltage pulses, which minimize the heating of the electrodes [Laroussi et al., 2007]. Currently, plasma jets are arguably the most widely used kind of source, and it can only be used for the treatment of bacteria when there is direct contact between the target and the jet. Plasma jets have the advantage of being easy to maintain and have a simple design. They do, however, only manage to cover a limited target region [Das et al., 2023b] [Nishime et al., 2017]. Since the

short-lived species like $\bullet\text{OH}$, which has the highest oxidation potential, vanishes quickly, the distance between the target pathogens and NTPs source becomes important. On the other hand, the pass-through process is ineffective in decontaminating the indoor environment and requires a long sterilization time. Hence, there is a need for an innovative method to generate active radicals at the site of contamination to eliminate ESKAPE bacteria and, therefore, reduce the risk of HAIs within confined spaces.

In this chapter, we examine the effectiveness of an in-house developed non-equilibrium cold plasma source in eradicating ESKAPE bacteria through an indirect treatment approach. The antibacterial efficacy of the NE-AACP source was tested in a controlled environment, with a 100 cm distance maintained between the target pathogens and the NE-AACP source. To our knowledge, this is the first study to utilize a cold plasma source for the indirect treatment of ESKAPE pathogens. The presence of active air ions and $\bullet\text{OH}$ was confirmed using an air ion counter and spectrophotometry, respectively. To mitigate the potential impact of O_3 on microorganism inactivation, a catalyst coating was employed. Additionally, this study explores how the active air ions and $\bullet\text{OH}$ generated by atmospheric cold plasma affects the inactivation mechanisms of ESKAPE pathogens.

6.2 Characteristics of Power Source

An already described co-axial DBD plasma source (section 2.2.1 in chapter 2) has been employed for this study. All the experiments were conducted in a 1000 ft³ (10 ft. × 10 ft. × 10 ft.) room where the temperature and RH were kept identical throughout at (23±2) °C and (50±5) %, respectively. A schematic representation of different elements of the complete experimental setup is shown in Figure 2.5. All the experiments were performed with a variable bipolar pulsed power supply [1 – 25 kV (peak voltage), 5 – 50 kHz (pulse repetition frequency), peak current maximum up to 1 A and 2-μsec pulse width].

The NE-AACP source has been operated with an operational time (T_{on}) and shutting time (T_{off}) of 2 μs and 48 μs, respectively. At a pulse repetition frequency (PRF) of 20 kHz, the duty ratio of the waveform is 0.04 (duty cycle ~ 4%). A typical voltage and current waveform of the NE-AACP source is shown in Figure 6.1. During every positive half cycle, especially during the rising pulse of the applied voltage, several micro-discharges are initiated. This results in a current with a positive polarity. Charges build up on the dielectric material's surface throughout this process, which results in a negative polarity current during the positive half cycle falling pulse. At an applied voltage (V) of 4.0 kV, the total current (I) was 143.6 mA, and the electric power dissipated in one duty cycle of the discharge period ($P_d = \text{duty ratio} \times \frac{1}{T} \int_0^T V I dt$) [Adhikari et al., 2020] is found to be 0.13 J/s.

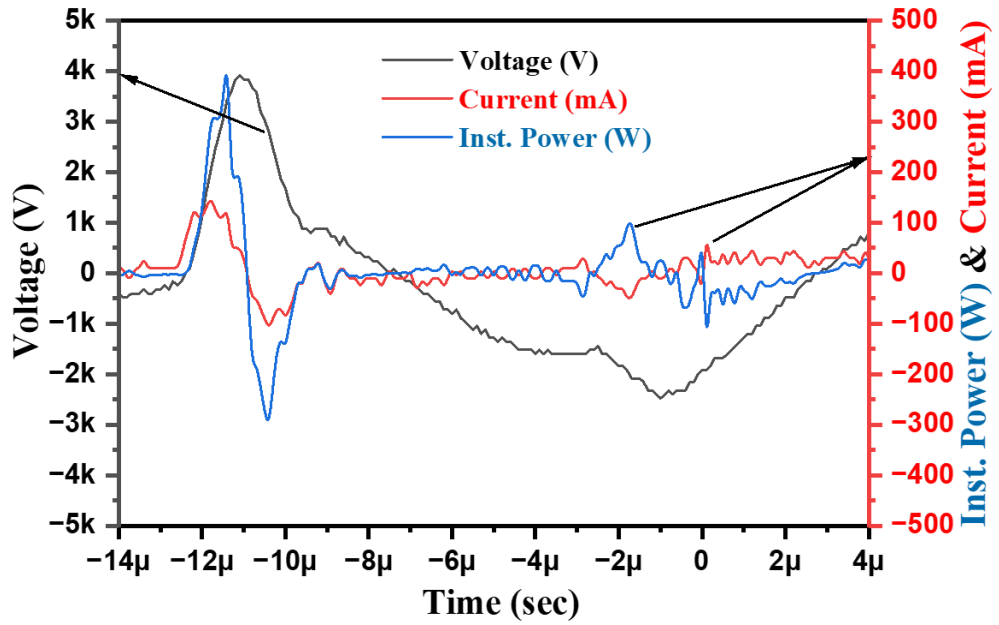


Figure 6.1: Typical voltage-current waveforms and instantaneous power vs time graph of the NE-AACP source.

6.3. Experimental Analysis and Discussion

6.3.1. Electrical Characteristics of NE-AACP Source

The combined effect of periodic localised heating and cooling of the neutral gas during plasma "on" and "off" durations, respectively, may be responsible for the production of non-thermal plasma. Ohmic heating, or the transfer of energy from HEEs to neutral atoms via elastic collisions, is the primary mechanism in atmospheric pressure plasmas that generate gas heating. This process increases the temperature of the neutral gas. The applied pulse in the present experiments has a total duration of $50\ \mu\text{s}$ (for a 20 kHz PRF). Consequently, the applied pulse power remains on for $2\ \mu\text{s}$ (T_{on}) and off for $48\ \mu\text{s}$ (T_{off}) at a 4% duty cycle. Figure 6.2 (a) shows an example of a pulse-modulated voltage waveform that illustrates the same. The source might be able to run for longer periods without overheating the electrodes if there was a longer interval between successive short-duration discharges. Repeated bipolar pulses create pulsed plasma, which in turn cause alternating sequences of cooling (plasma contraction) and heating (gas expansion) during the "on" and "off" phases, respectively. Enhanced depletion and localised heating of the neutral gas in the annular discharge zone is caused by an increase in electron generation surrounding the powered electrode (by electron impact ionisation) during the bipolar pulse "on" duration. But, the plasma quenches in the "off" duration, and the gas cools down. Due to the nearly identical duration of the "on" and "off" periods of an AC power source, gas/device heating is a significant issue [Brandenburg, 2018]. The current study dealt with this problem by employing a bipolar pulsed power source with an extended shutting-off period.

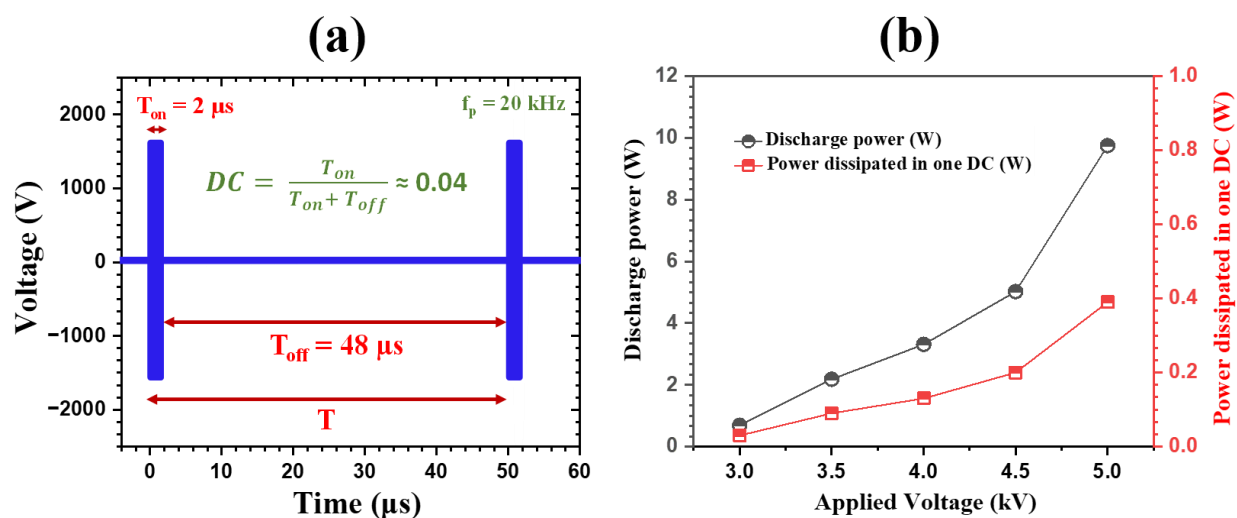


Figure 6.2: (a) Typical waveform of pulse power source at 20 kHz PRF and 4% duty cycle; (b) Discharge power dissipated in the NE-AACP source and the power consumed in one duty cycle at various applied voltages.

The power dissipated in the NE-AACP source, and the power consumed in one duty cycle at various applied voltages are shown in Figure 6.2 (b). It can be observed from this figure that with increasing applied voltage, the corresponding power dissipated in discharge increases from 0.69 to 9.75 W, as the voltage is changed from 3.0 to 5.0 kV at a fixed frequency of 20 kHz. However, the power dissipated in one DC is very low and found in the range of 0.03 – 0.4 W for the applied parameters. For example, in the case of 4 kV/20 kHz, the electric energy per pulse is found to be 1.65×10^{-4} Joule, which corresponds to a 3.31 W average discharge power consumption in a NE-AACP source, but the power consumed in plasma discharge formation is only 0.13 W. This low power consumption is key for the sustenance of plasma discharge and consequently reduce device heating.

6.3.2 UV Intensity and TiO₂ Activation Mechanism

While it is true that NTPs can produce UV radiation [Mei et al., 2016a] [Zhang et al., 2017], but the photon flux it emits is insufficient to have a meaningful impact on the activation of TiO₂ catalysts, i.e., extensively documented and proven in multiple studies [Mei et al., 2016a] [Chen et al., 2017]. In a study conducted by Sano et al. [Sano et al., 2006b], at an input power of 5 W, the overall UV intensity in an atmospheric air-surface discharge was measured to be only 2.5 $\mu\text{W}/\text{cm}^2$. This resulted in a relatively weak photocatalysis effect, contributing less than 0.2% to the acetaldehyde degradation by the plasma itself. Mei et al. [Mei et al., 2016a] demonstrated the importance of a physical phenomenon in a DBD plasma, specifically the electric field amplification caused by inserting TiO₂ pellets into the discharge gap. This activation of photocatalytic processes facilitates the dissociation of CO₂. According to Whitehead et al. [Whitehead, 2010] [Wallis et al., 2007], in a plasma catalysis system, electrons with an energy greater than 3.0 eV can generate pairs of negatively charged electrons (e^-) and positively charged holes (h^+) on the surface of rutile TiO₂. This process is similar to the action of UV excitation. The generation of $e^- - h^+$ pairs, which enables the subsequent redox reaction, is regarded as the first and most important step in photocatalytic processes.

The UV intensity produced by the NE-AACP source has found to be very low ($\sim 5 - 17 \mu\text{W}/\text{cm}^2$) at various applied parameters [see Figure 6.3 (a)]. It is to be noted that this value is much lower than the UV intensity produced by conventional UV lamps ($\sim 10 - 40 \text{ mW}/\text{cm}^2$) to activate the photocatalysts in standard photocatalytic reactions. Under the influence of HEEs generated by the NE-AACP source, Figure 6.3 (b) illustrates the possible activation mechanism of TiO₂. The electrons and holes generated by chemical processes react with the oxygen and water

molecules in their vicinity, forming $O_2^{\bullet-}$ and $\bullet OH$. After a series of reactions, the $O_2^{\bullet-}$ will subsequently generate $\bullet OH$. These $\bullet OH$ acts as an oxidizing agent and changes toxic biological/organic compounds -into CO_2 , H_2O and other innocuous by-products.

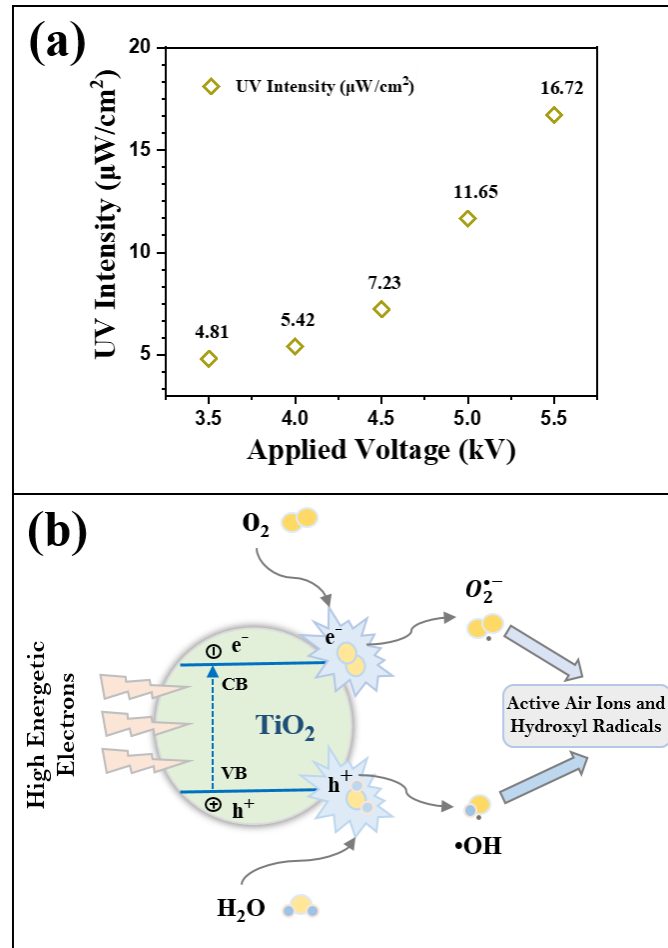


Figure 6.3: (a) UV intensity produced by the NE-AACP source; (b) Activation mechanism of TiO_2 catalyst for the generation of active air ions and $\bullet OH$.

6.4. Active Air Ions and Reactive Species

6.4.1. Negative and Positive Air Ions

The plasma-produced HEEs (> 3.2 eV) collide with the TiO_2 nanoparticle catalyst leading to the generation of electron-hole pair via reaction: $TiO_2 + e^- \rightarrow TiO_2(e_{CB}^- + h_{VB}^+)$. Electrons in the CB can decrease the O_2 and generate superoxide ions ($O_2^{\bullet-}$). The interaction between $O_2^{\bullet-}$ and holes in the VB results in the formation of singlet oxygen ($^1O_2$). The VB holes react with surface adsorbed H_2O or OH^- , leading to the production of $\bullet OH$ [see Figure 6.3 (b)]. Furthermore, these electrons and holes collide with the different molecules present in the air, leading to the generation of NAI and PAI. The concentration of NAI and PAI are accordingly measured at a distance of 100 cm from NE-AACP source under different power settings, which were varied between $500 - 1.2 \times 10^5$ ions/cc and $500 - 1.5 \times 10^5$ ions/cc, respectively (see Figure 6.4). According to the WHO, clean air is defined as having concentrations of NAIs and PAIs of greater than 1000 and 1200 ions/cc, respectively [Li et al., 2022]. We are able to effectively generate active ions (negative and positive) in the indoor environment, similar to Mother Nature. The optimum concentration of NAIs and PAIs are $\sim 18,500$ ions/cc and $\sim 25,500$ ions/cc, respectively, that distributes to ~ 1000 ions/cc and ~ 1200 ions/cc in an enclosed environment of 28.3 m³, at a

discharge power of 3.31 W. Accordingly, all the further experiments have been performed at an optimized power of 3.31 W.

In the cold air plasma discharge, the most common NAIs and PAIs that are present include NO_2^- , NO_3^- , HO_2^- , $N_2O_2^-$, $N_2O_3^-$, etc and NO^+ , N_2^+ , O_2^+ , O^+ , OH^+ , etc. [Das et al., 2023b] [Das et al., 2023a]. These active air ions and species interact with ambient air and water molecules present in the environment and generate $\bullet OH$ and other reactive species (at the site of contamination) by following equations (6.1) – (6.2) of Table 6.1.

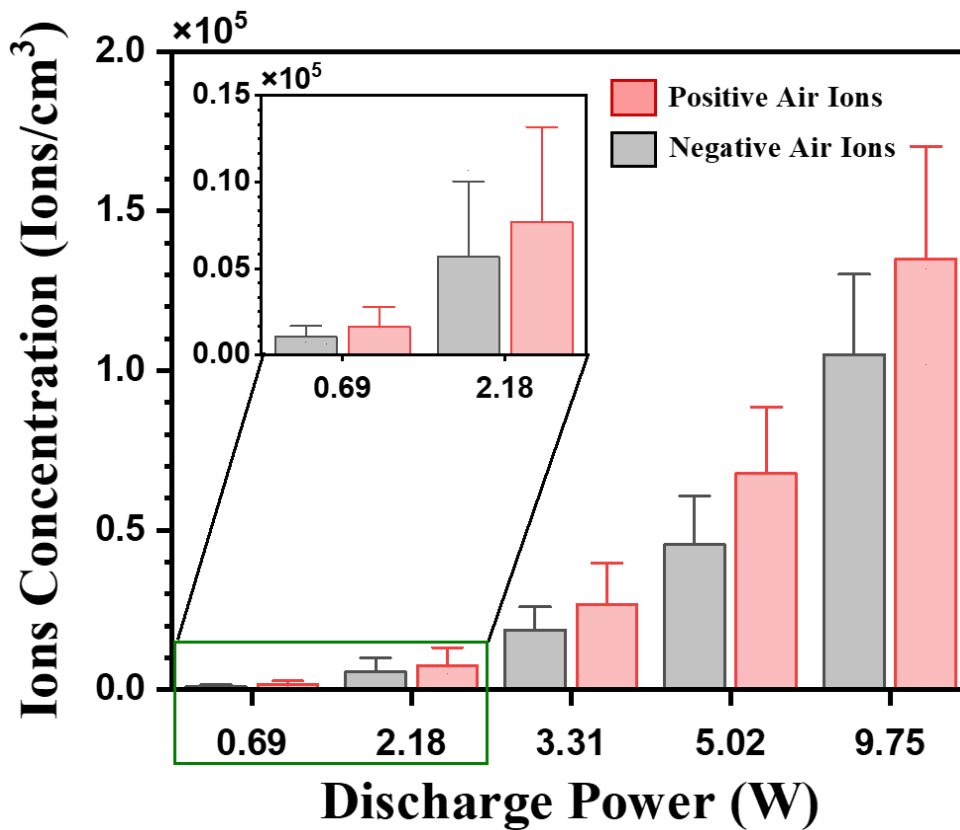


Figure 6.4: Concentration of NAIs and PAIs at a distance of 100 cm from NE-AACP source at different power settings.

Table 6.1. Reaction mechanism of formation of $\bullet OH$ and other reactive species in ambient air.

Reaction	Equation nos.
$e^- + O_2 \rightarrow O + O + e^-$	(6.1)
$e^- + N_2 \rightarrow N + N + e^-$	(6.2)
$e^-(TiO_2) + H_2O \rightarrow \bullet H + \bullet OH + e^-$	(6.3)
$O + H_2O \rightarrow 2 \bullet OH$	(6.4)
$O + O_2 \rightarrow O_3$	(6.5)
$e^-(TiO_2) + O_2 \rightarrow O_2^-$	(6.6)
$N_2^* + H_2O \rightarrow \bullet H + \bullet OH + N_2$	(6.7)
$h^+(TiO_2) + OH^- \rightarrow \bullet OH$	(6.8)
$O_2^- + 2H^+ \rightarrow 2 \bullet OH$	(6.9)

$h^+(TiO_2) + H_2O \rightarrow \bullet OH + H^+$	(6.10)
$H_2O_2 + O_2^- \rightarrow \bullet OH + OH^- + O_2$	(6.11)

Fletcher et al. [312] demonstrated the bactericidal effects of active air ions and suggested that the role of these ions alone in bactericidal action might be overestimated. They indicated that additional strong oxidizing agents, such as $\bullet OH$, are necessary to enhance the efficacy of plasma sources against microorganisms in enclosed environments. To address this, we applied TiO_2 nanoparticles (NPs) to the outer electrode in our study. The amount of $\bullet OH$ generated was identified and quantified using optical emission spectroscopy (OES) and spectrophotometry, respectively.

6.4.2. Hydroxyl Radicals ($\bullet OH$) and Ozone (O_3)

To quantify the concentration of $\bullet OH$, the TA solution has been exposed to the NE-AACP source. Fluorescence spectra of TA solution for different treatment durations have been recorded and then compared with the pre-calibrated fluorescence curve of HTA. Although $\bullet OH$ has a short lifetime, it forms HTA after reacting with TA, which is notably stable and possesses a longer lifetime, thereby allowing its detection through fluorescence spectra. As time progressed, the fluorescence intensity increased (see Figure 6.5), signifying the accumulation of more $\bullet OH$ captured by TA. Figure 6.6 (a) depicts the time-dependent concentration of $\bullet OH$ in the case of with and without TiO_2 coating. The measurements are taken at 100 cm from the NE-AACP source. In the case of TiO_2 coating, the concentration of $\bullet OH$ is around 14.15 μM after 10 minutes of exposure and continues to increase to 73.11 μM after 60 minutes. However, in the case of bare electrodes, the concentration of $\bullet OH$ is around 7.41 μM and 53.55 μM after 10 minutes and 60 minutes of exposure, respectively. The difference mainly arises from the generation of electron and hole pairs by the TiO_2 NPs. The transfer of electrons from the CB and holes from the VB of the photocatalyst enables water and oxygen molecules to generate NAIs through a series of chain reactions [Russo et al., 2020]. As reported by Jiang et al. [Jiang et al., 2018], the lifetime of these NAIs was found to be 100 seconds to several minutes. Luts and Salm [A. Luts and J. Salm, 1994] identified that O_2^- is the primarily negatively charged ion and is more stable than other NAIs. These NAIs have been utilized for the on-site production of $\bullet OH$ (equation 6.9) and other reactive species. The reactive species, including $\bullet OH$ and O_3 , have been demonstrated in numerous studies to be crucial in the inactivation of microorganisms [Sakudo et al., 2019]. It is well-known that $\bullet OH$ has a strong oxidation potential of 2.8 eV, greater than the oxidation potential of other reactive species. The $\bullet OH$ can act directly with the microorganisms, including ESKAPE pathogens; therefore, they play an important role in the removal of pathogens present on different surfaces.

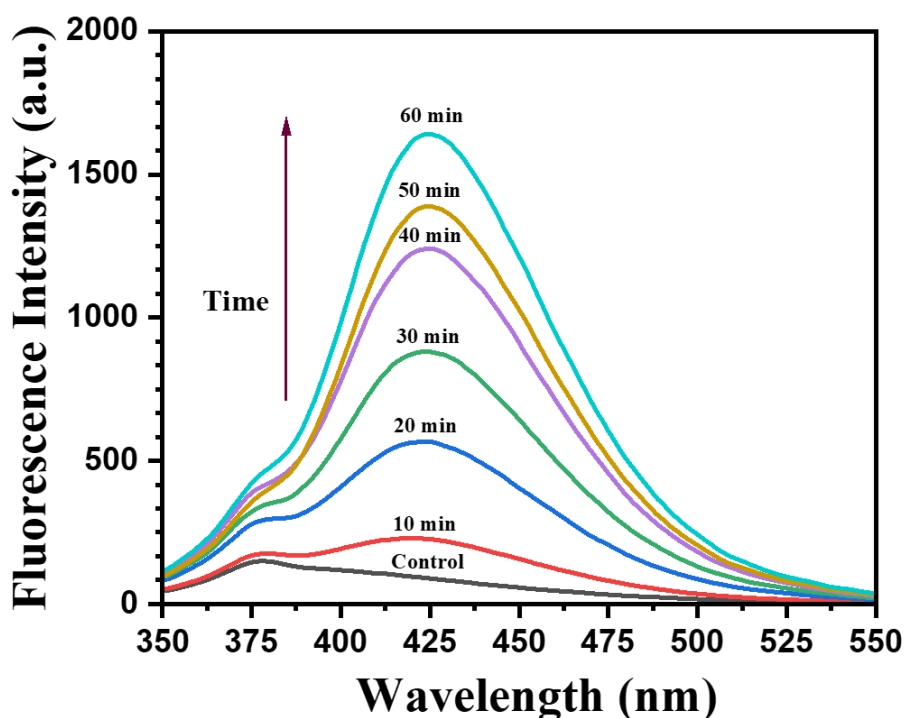


Figure 6.5. Fluorescence spectra of TA probe exposed to the NE-AACP source.

Additionally, we looked at the O_3 concentration's temporal change in the room where the NE-AACP source was operating. The concentration of O_3 increased when the NE-AACP source was in operation mode, and then it reduced when it was turned off as the O_3 decomposed, and the room's concentration reduced to 0 ppm. The concentration of O_3 in the room increases when the plasma source is turned on until an equilibrium state is reached, where the amount of O_3 produced in the DBD discharge is equal to the amount of O_3 being decomposed, primarily through unimolecular decay and recombination in collisions with air molecules [Jian et al., 2022] [Batakliiev et al., 2014]. The O_3 concentration starts dropping when the NE-AACP source is turned off because decomposition process become dominant. After the plasma source was turned on, the equilibrium condition was obtained approximately 1.5 hours later [see Figure 6.6 (b)].

We have measured the O_3 concentration during plasma discharge in case of with and without TiO_2 coating at a distance of 30 cm due to the antimicrobial properties of the produced O_3 . Given its detrimental impact on human health, O_3 has been targeted to be removed from DBD plasma-based air cleaning technologies. In the case of TiO_2 coating, plasma discharge increased the O_3 level to 0.13 ppm, which reduced to its original level when the plasma had been turned off [see Figure 6.6 (b)]. On the other hand, while using a bare electrode, the O_3 concentration experienced a slight increase, reaching a level of 0.20 ppm. The variation in O_3 concentration can be attributed to the high dielectric constant of TiO_2 nanoparticles (40 – 60). As a result, the breakdown of O_3 produced in the plasma is thought to be caused by the strong electric field that is created at the contact points [Abd Allah et al., 2014].

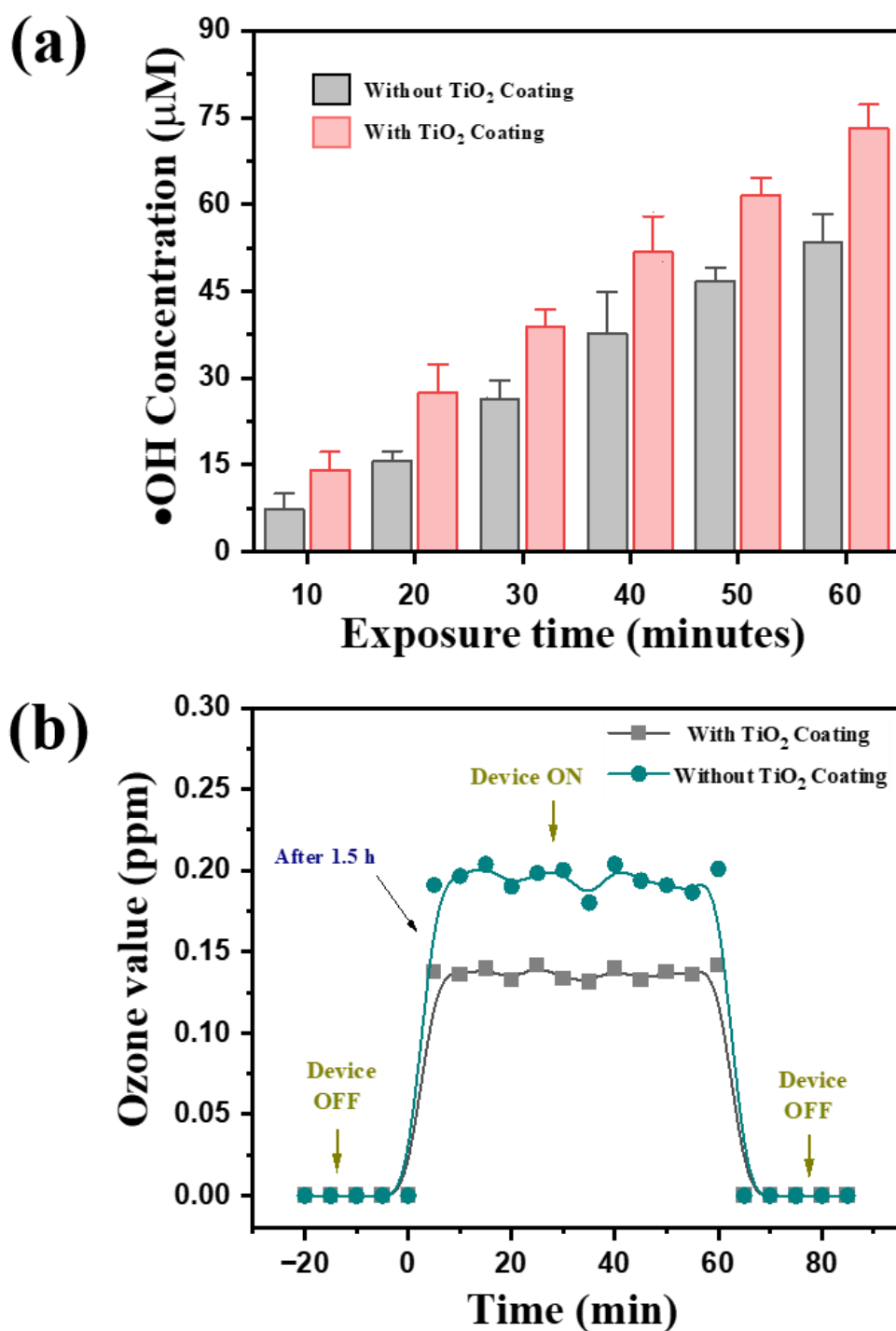


Figure 6.6: (a) Temporal concentration of $\bullet\text{OH}$ with and without TiO_2 coating at an optimized discharge power of 3.31 W; (b) concentration of O_3 in different scenarios.

The allowable O_3 concentration levels are set at a time-weighted average of 0.1 ppm over 8 hours and a short-term exposure limit of 0.3 ppm for a 15-minute interval [Kim et al., 2020]. Operating the NE-AACP device in an enclosed environment for over 1 hour resulted in a time-weighted average O_3 concentration of around 0.13 ppm. This level of O_3 is well below international safety norms. The TiO_2 coating reduces O_3 concentration significantly, but the concentration of active air ions and OH radicals increased further, which participates in the eradication of ESKAPE pathogens.

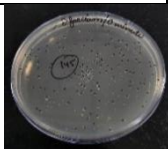
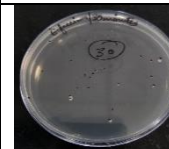
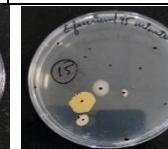
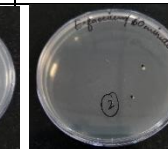
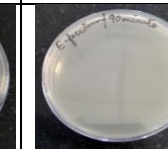
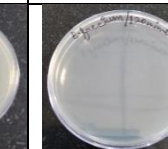
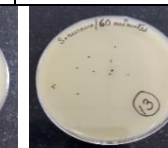
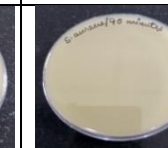
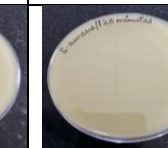
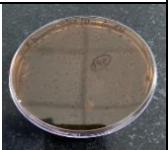
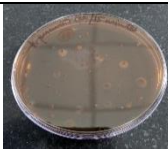
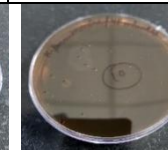
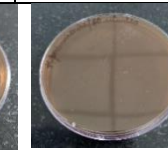
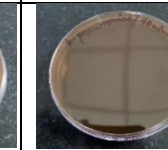
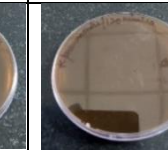
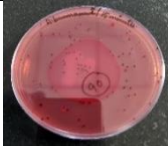
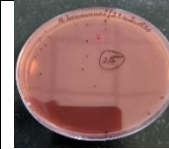
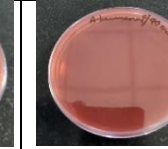
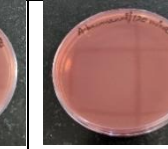
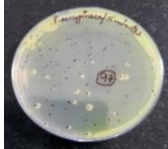
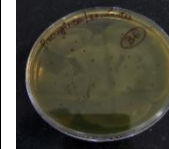
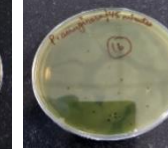
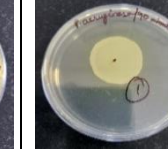
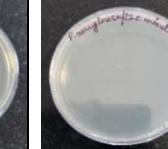
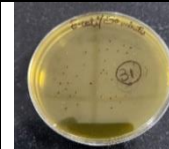
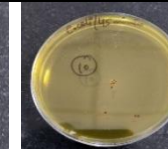
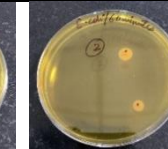
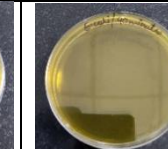
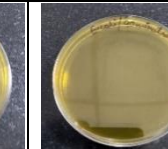
6.5. Antibacterial Properties Evaluation of NE-AACP Source

This section presents the findings of a comprehensive antibacterial efficacy study that aimed to assess the effectiveness of the in-house developed NE-AACP source as a tool to prevent and control infections caused by ESKAPE pathogens. The impact of varying treatment durations on the inactivation of ESKAPE bacteria has been investigated, as illustrated in Table 6.2. The images illustrate ESKAPE bacteria that have been grown on specified agar after incubation, following the application of various treatment durations to the inoculated suspensions. The corresponding quantitative measurements of the CFUs are also mentioned on each agar plate. The control samples exhibited a higher number of colonies compared to the treated ones. After 15 minutes of NE-AACP treatment, the number of colonies decreased substantially and continued to decrease with the increasing treatment time.

The destruction of bacteria was observed to be associated with exposure to the NE-AACP. Figure 6.7 (a – f) shows the reduction in CFU/plate for ESKAPE bacteria determined using standard plate count. Overall, longer exposure times have been required to eradicate *A. baumannii*, *P. aeruginosa*, and *E. coli* than *E. faecium*, *S. aureus*, and *K. pneumoniae*. The plasma treatment time required for inactivating both types of bacteria vary due to their distinct structures. Gram-positive bacteria, such as *K. pneumoniae*, have a thick peptidoglycan cell wall whereas gram-negative bacteria, such as *P. aeruginosa*, possess a thin peptidoglycan cell wall. In addition to thin peptidoglycan cells, they have an outer membrane that contains lipopolysaccharides surrounding the cell. That is the reason why gram-negative bacteria are often termed diderms and gram-positive bacteria are termed monoderms. The diderms structure can make them more resistant to certain antibiotics and disinfectants compared to gram-positive bacteria (*E. faecium* and *S. aureus*). [Ahlawat et al., 2024a] [Mai-Prochnow et al., 2016]. The complete inactivation of *S. aureus* and *K. pneumoniae* takes only 45 minutes of treatment, while the complete inactivation of other ESKAPE pathogens requires around 60 minutes. When it comes to deactivating ESKAPE pathogens, the indirect treatment method of the NE-AACP source has proven to be highly effective.

Westover et al. [Westover et al., 2023] employed a substantially high O₃ concentration of 20 ppm to eliminate *E. coli* bacteria. They observed a 99% reduction in viable bacteria after a 2-hour treatment cycle. According to international guidelines, this O₃ concentration is excessively high to be acceptable in confined environments. Nevertheless, the O₃ concentration generated by the NE-AACP source is extremely low (0.07 ppm), which is insufficient for effectively neutralising the ESKAPE pathogens. Fletcher et al. [Fletcher et al., 2007] demonstrated the bactericidal action of active air ions and showed that the bactericidal action of only active air ions may have been overestimated. Hence, in this investigation, the •OH generated via chain reaction at the site of target pathogens is the main species accountable for the inactivation [Ahlawat et al., 2022].

Table 6.2. Growth of ESKAPE bacteria on agar after subjecting the suspension to NE-AACP source at different exposure times.

Type of Bacteria/ Treatment time (min)	0	15	30	45	60	90	120
<i>E. faecium</i>							
<i>S. aureus</i>							
<i>K. pneumoniae</i>							
<i>A. baumannii</i>							
<i>P. aeruginosa</i>							
<i>E. coli</i>							

The inset in Figure 6.7 (a – f) shows the inactivation percentage of ESKAPE bacteria when exposed to the active air ions along with the $\bullet\text{OH}$. Complete inactivation was observed in just 60 minutes of treatment, showing the antibacterial efficacy of the developed NE-AACP source. A significant reduction in the bacterial load with increasing cold plasma exposure time might be attributed to extended interactions of the produced active air ions and $\bullet\text{OH}$ with the target pathogens. The obtained results are consistent with the earlier studies performed to eradicate ESKAPE pathogens [Scholtz et al., 2021] [Flynn et al., 2015b] [Martines, 2020]. When utilized as an air disinfectant (that employs active air ions and OH radicals-based disinfection), the developed NE-AACP source has been shown to be harmless to humans and the environment. Because of this, it can be continually utilized when humans are around to stop the growth of pathogenic microorganisms in indoor air.

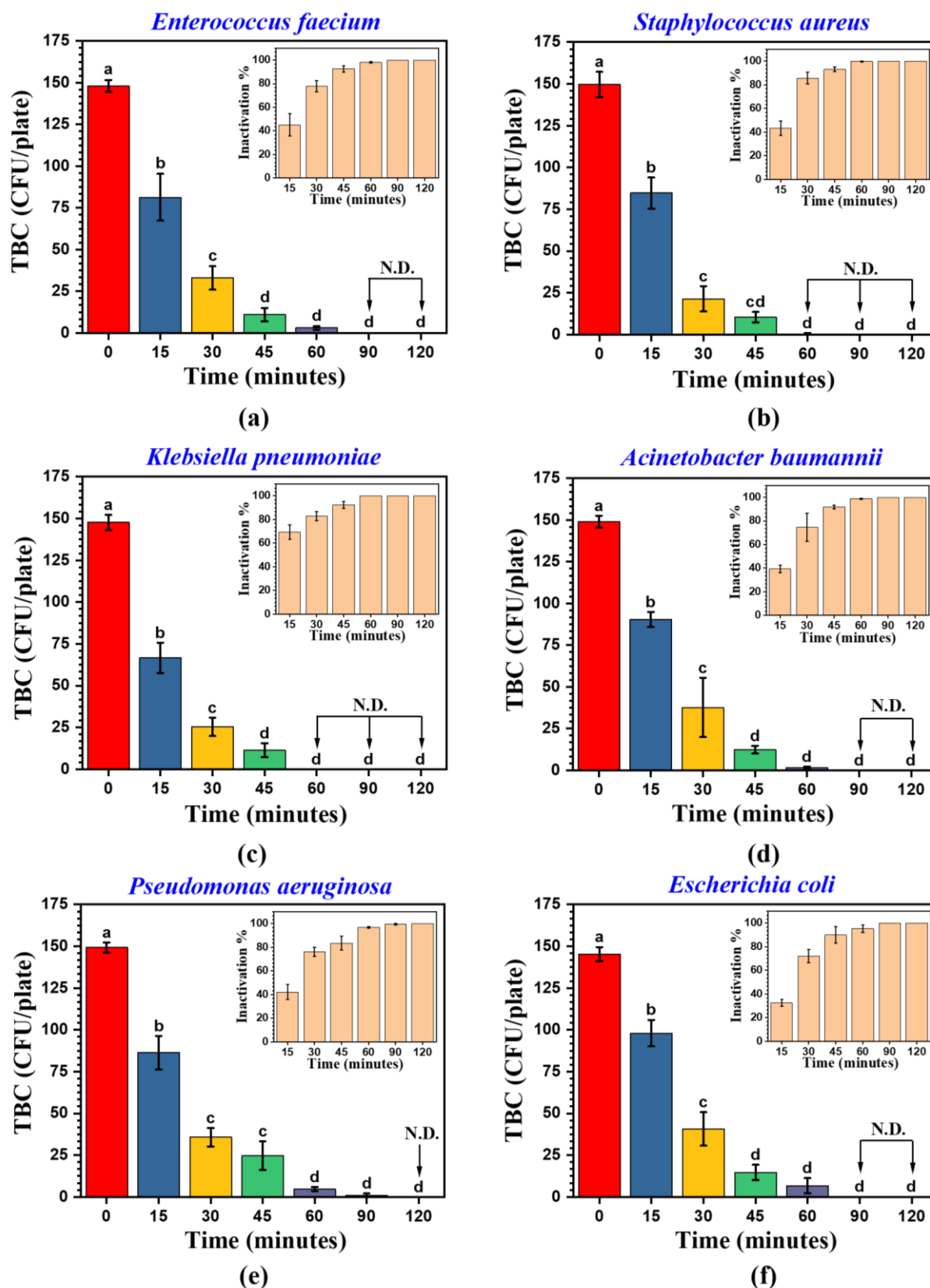


Figure 6.7: Reduction in CFU/plate and inactivation percentage for ESKAPE bacteria determined using standard plate count: (a) *Enterococcus faecium*; (b) *Staphylococcus aureus*; (c) *Klebsiella pneumoniae*; (d) *Acinetobacter baumannii*; (e) *Pseudomonas aeruginosa*; (f) *Escherichia coli*. Bars represent the standard deviation from the mean.

6.6 Bacterial Inactivation Mechanism

In the NTPs discharges, a cocktail of species such as electrons, ions, charged particles, RONS, along with UV radiation and electric field, play a synergistic role in bacterial inactivation mechanisms [Das et al., 2023b] [Das et al., 2023a]. The NE-AACP produced active air ions and reactive species, especially $\bullet\text{OH}$, which is mainly responsible for the inactivation of pathogenic microorganisms and NTPs interaction with the living matter.

The possible mechanisms of microbial inactivation are as follows:

1. ROS produced by the NTP discharges causes lipid peroxidation, primarily in fatty acids close to the cell surface. At the same time, $\bullet\text{OH}$ is primarily responsible for the chemical alteration and destruction of proteins [Martines, 2020] [Čtvrtečková et al., 2019].
2. Mechanical cell damage, specifically electrostatic disruption triggered by the electrostatic forces caused by the charged particles accumulated on the cells. The electroporation from the charged particles' bombardment is also responsible for cell damage [Liao et al., 2017]. This is because of positive ion sheath formation on negatively charged bacterial cell walls [Stoffels et al., 2008]. Within the microsecond ion response time scale, the electrostatic stress would surpass the internal resistive force of the bacteria. This would compromise the cell's structural integrity and cause membrane permeabilization or poration as well as the disintegration of the cell wall.
3. The penetration of active air ions and reactive species into the cell through membrane voids would lead to the destruction of intracellular components, such as DNA denaturation and protein oxidation.
4. On the other side, the produced positive and negative air ions coexist in the atmosphere. When they adhere to the surfaces of airborne bacteria, they undergo a chemical reaction that results in the production of $\bullet\text{OH}$ and H_2O_2 , which remove hydrogen atoms from the bacteria's cells and ultimately cause their death. Thus, by means of oxidative and electrostatic stress, the detected gas phase molecules might contribute to the inactivation of bacteria.

A simplified inactivation/killing mechanism of bacteria by NE-AACP generated reactive species is shown in Figure 6.8.

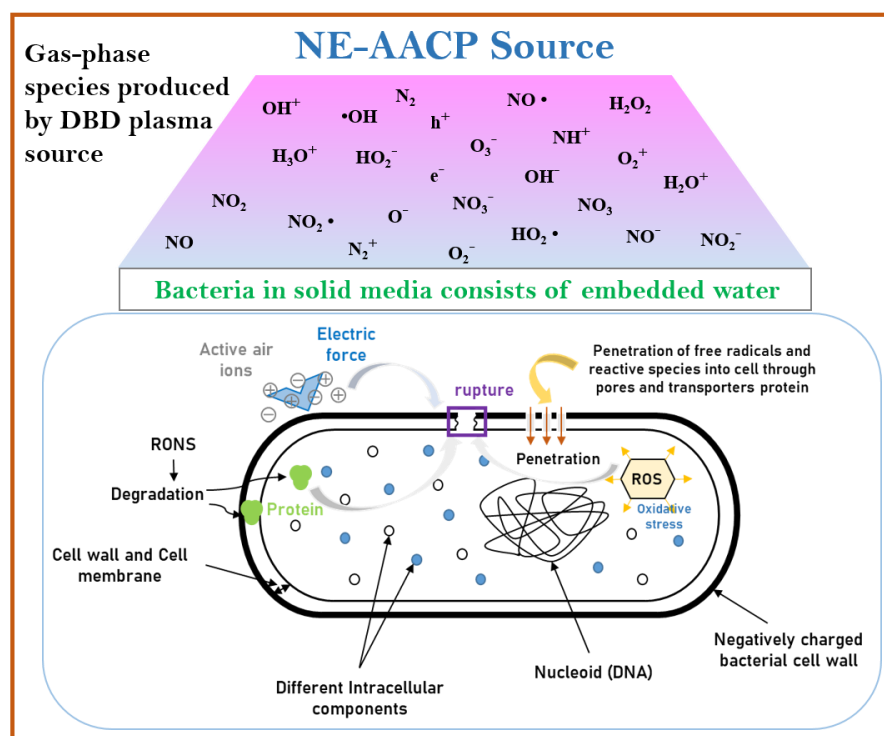


Figure 6.8: Schematic diagram showing the effects of NE-AACP generated reactive species on the inactivation/killing of a bacterial cell.

6.8 Conclusion

This study distinguishes itself from previous research by focusing on the immediate effects of bipolar pulsed-powered non-equilibrium atmospheric cold plasma (NE-AACP) on the removal of ESKAPE pathogens through active air ions and $\bullet\text{OH}$. Bipolar pulsed-powered DBD plasma offers advantages over AC, DC, RF, and microwave plasma due to its capability to deliver energy in short, high-voltage pulses, which minimizes electrode heating. The study confirms that NE-AACP effectively inactivates ESKAPE bacteria in indoor environments. The results indicate that NE-AACP is a promising disinfection method for protecting individuals from ESKAPE bacteria, which are commonly associated with healthcare-associated infections (HAIs). Aerosol inhalation from coughing and sneezing or contact with droplet-contaminated surfaces are recognized as primary modes of transmission for respiratory pathogens, including bacteria and viruses [Assadi et al., 2022] [Wang et al., 2021a]. When used as an air disinfectant, the NE-AACP source –employing active air ions and $\bullet\text{OH}$ radicals –has been demonstrated to be safe for humans and the environment, allowing for continuous use even when people are present. This helps prevent the accumulation of pathogenic microorganisms in indoor air. Moreover, the O_3 concentrations produced during NE-AACP operation are within international safety standards [Weschler, 2000].

Most studies on the inactivation of ESKAPE bacteria using NTP technologies have focused on direct treatment methods, where microorganisms are exposed directly to plasma. While these strategies are highly effective at rapidly eradicating ESKAPE bacteria, they typically cover only a small area and are less effective for larger regions or when the pollutants are not in direct contact with the plasma. In contrast, the current study explores an indirect treatment approach, assessing the impact of active air ions and hydroxyl ($\bullet\text{OH}$) radicals generated by the NE-AACP on ESKAPE pathogens. For this investigation, control samples of ESKAPE bacteria were placed 100 cm from the NE-AACP source. The results demonstrated substantial efficacy achieving 99.9% inactivation of ESKAPE bacteria within a 60-minute treatment period.