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REVIEW ARTICLE

Bactericidal Effects of Plasma Induced Reactive Species in Dielectric Barrier Gas–Liquid Discharge

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Abstract An atmospheric helium dielectric barrier discharge is used to treat *Staphylococcus aureus* (*S. aureus*) to study plasma bacteria inactivation in the gas–liquid phase. Optical emission spectroscopy, mass spectrometry, and spectrophotometry are used to analyze the products induced by the plasma in the liquid as well as interactions between the liquid products and bacteria. The bactericidal mechanisms associated with the liquid products and different treatment protocols are investigated. The short-lived reactive species produce efficient inactivation effects in the direct plasma treatment and long-lived reactive species show continuous residual bactericidal effects. Additionally, even the minor initial damage caused by direct plasma exposure promotes the residual inactivation effect. After the discharge treatment for 1, 3, 5, and 8 min, the initial damage causes residual bacterial inactivation of 9.3, 37.2, 81.8, and 86.7%, respectively. Meanwhile, the discharge time and

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storage time in the different treatment processes can be optimized to achieve better bactericidal efficiency and energy efficiency. The results provide insights into the future development of plasma medicine and water purification.

Keywords Atmosphere dielectric barrier discharge plasma · Bacteria inactivation · Bactericidal efficiency · Initial damage · Reactive species

Introduction

Boasting advantages such as generation of reactive species as well as flexible room-temperature operation, cold plasmas are widely applied to bacteria inactivation, induction of apoptosis, living tissue treatment, water treatment, and materials modification [1–22]. In particular, atmospheric-pressure cold plasmas have attracted increasing attention because they can effectively eradicate microorganisms such as spores and biofilms and useful in the fields of medical instrumentation, food sanitation, and textile. However, bacteria can survive under humid conditions and so inactivation of bacteria in solutions is important. In a liquid, the energetic particles created by the plasma are trapped by the liquid and cannot interact with bacteria effectively. However, energetic particles can produce reactive species in water and it is generally agreed that short-lived reactive species such as superoxide (O_2^-), hydroxyl radical (OH·), and singlet oxygen and long-lived reactive species (RS) including H_2O_2 and O_3 are significant inactivation agents, while other plasma-generated components such as heat, UV photons, and high electric fields make minor contributions to inactivation of bacteria [23–25]. Therefore, it is crucial to fathom the roles and mechanisms of reactive species in bacteria inactivation of bacteria in gas–liquid plasmas.

Plasma–liquid interactions and the related bactericidal action have been investigated by experimental and numerical model methods. For example, Wang et al. [26] used a custom-made atmospheric dielectric barrier discharge reactor to kill *S. japonicum cercariae* using He, O_2, and air as feeding gases and observed that reactive oxygen species such as O atoms abundant in the O_2 plasma and NO in the air plasma played major roles in killing *S. japonicum cercariae* via oxidation mechanisms. Oehmigen et al. [27] discussed the synergistic action of reactive oxygen and nitrogen species responsible for the antimicrobial effects in the surface dielectric barrier discharge plasma. Lukes et al. [28, 29] demonstrated that an electrical discharge plasma in water and at the gas–liquid interface produced chemical and biological effects and induced various kinds of reactive species in the liquid. Lu et al. studied the propagation mechanism of plasma jets and biomedical applications by the two-dimensional numerical model and key equations of plasma physics and chemistry were introduced [30]. However, in spite of recent advances, more studies are needed. Firstly, the respective bacterial effects of long-lived and short-lived reactive species are still not clear. Secondly, the bactericidal efficiency associated with the liquid products and different treatment protocols have not been investigated extensively. Thirdly, during gas–liquid plasma inactivation, the reactive species in the liquid have inactivation effects on bacteria. The discharge treatment causes initial damage to bacteria and the role of initial damage in subsequent inactivation is unclear. Hence, more studies are needed to enhance the efficiency of atmospheric-pressure cold plasmas and provide better understanding of the plasma inactivation mechanism. In a low-temperature plasma, complex chemical and physical processes occur and various biological agents are generated in the gas–liquid environment. The processing parameters such as the initial concentration and treatment

protocols may produce different inactivation effects and their roles in plasma inactivation must be better understood.

In this work, the different discharge properties are determined and the reactive species produced by the plasma are detected by optical spectroscopy in the cases of pure helium and helium/water. Mass spectrometry is employed to provide the clues of chemical and physical processes in the liquid phase and spectrophotometry is conducted to determine the reactive species concentration in the liquid. The bacterial inactivation mechanisms related to the liquid products and different treatment methods are studied. Based on the different experimental processes, the different effects of long-lived and short-lived reactive species on bacteria inactivation are determined. The influence of different conditions such as the initial concentration of bacteria and exposure methods is studied and the role of initial damage in subsequent inactivation is studied.

Experimental Methods

Atmospheric-Pressure DBD Plasmas Source

The atmospheric-pressure DBD plasma apparatus are shown in Fig. 1a, b. There are four DBD plasma reactors in a hollow plexi-glass cylinder (reactor chamber) and four stainless

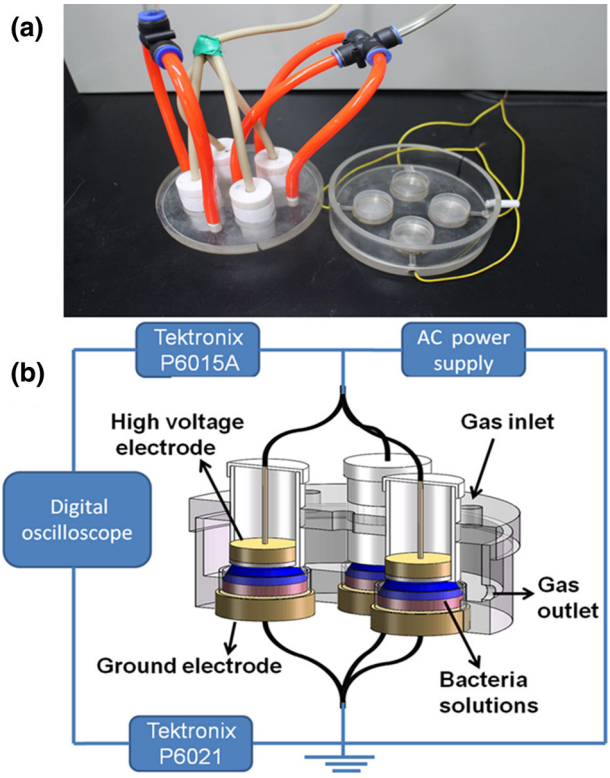

Fig. 1 **a** Photograph of the reactor chamber and **b** schematic diagram of the experimental setup in DBD plasma inactivation of *S. aureus*

steel cylinders 32 mm in diameter are used as the high voltage electrode covered by 1 mm thick quartz glass as the insulating dielectric barrier. A 37 mm diameter stainless steel cylinder serves as the grounded electrode. In the experiment, the discharge spacing between the bacterium suspension surface and bottom of the quartz glass was fixed at 5 mm and the apparatus was equipped with an alternating current power supply with continuous and tunable output voltages and frequencies. Helium (99.99% pure) was introduced at a flow rate of 80 standard liters per hour (SLH) through a gas inlet. In order to eliminate air from the reactor chamber as much as possible, the chamber was purged with helium for 5 min before the experiment. The applied voltage and current in the atmospheric-pressure helium DBD plasma were monitored by a Tektronix MSO 5104 digital oscill oscope with a 1000:1 high voltage probe (Tektronix P6015A) and current probe (Tektronix P6021). The gas–liquid plasma was generated at a voltage of 11.6 kV (peak to peak), frequency of 24 kHz, and power of 21 W.

Optical Emission Spectroscopy (OES)

The light emitted from the DBD plasma discharge was monitored by a spectrometer (AvaSpec-2048–8-RM) with a grating of 2400 grooves/mm at the wavelength range between 200 and 900 nm.

Mass Spectrometry (MS)

A molecular-beam mass spectrometer (MBMS, Hiden EQP mass/energy analyzer HPR 60) was operated in the time-averaged mode. The distance between the orifice of the mass spectrometer and bottom of the quartz glass of the DBD plasma was 5 mm.

Monitoring of RS in Deionized Water Induced by Plasma

The concentrations of long-lived RS such as hydrogen peroxide, ozone, nitrate, and nitrite in the DBD plasma-treated liquid were measured spectrophotometrically on the PhotoLab 6100 (WTW, Germany). The four relevant test kits were 18789, 00607, 09713, and 00609 respectively. The methods were in accordance with the manufacturer's manual and the same as that used by Shen et al. [31, 32].

Cultivation of *S. aureus* Suspensions

Staphylococcus aureus (*S. aureus*) was cultured in the Luria–Bertani (LB) broth for 12 h at 37 °C in an incubator. After culturing overnight, the concentration of *S. aureus* was approximately 10^9 CFU ml^{-1}. Two dilutions ten times each reduced the bacteria concentration to 10^7 CFU ml^{-1}. The sample was treated by four processes: direct discharge treatment, direct treatment with soaking, indirect treatment with soaking, and direct discharge treatment followed by centrifugation and resuspension in water with the same volume as shown in Fig. 2. The bacteria suspension was prepared by adding 30 µl of the initial concentration bacteria to 3 ml of deionized water. In the direct treatment process (a), 3 ml of the bacteria suspension were put on four 35 mm diameter dishes and exposed to the DBD plasma for 1, 3, 5, and 8 min, respectively. In the second process (b), 30 µl of the initial concentration bacteria were added to 3 ml of deionized water. The bacteria suspensions were directly treated for 5 min and soaked for 40 min. In the third process (c),

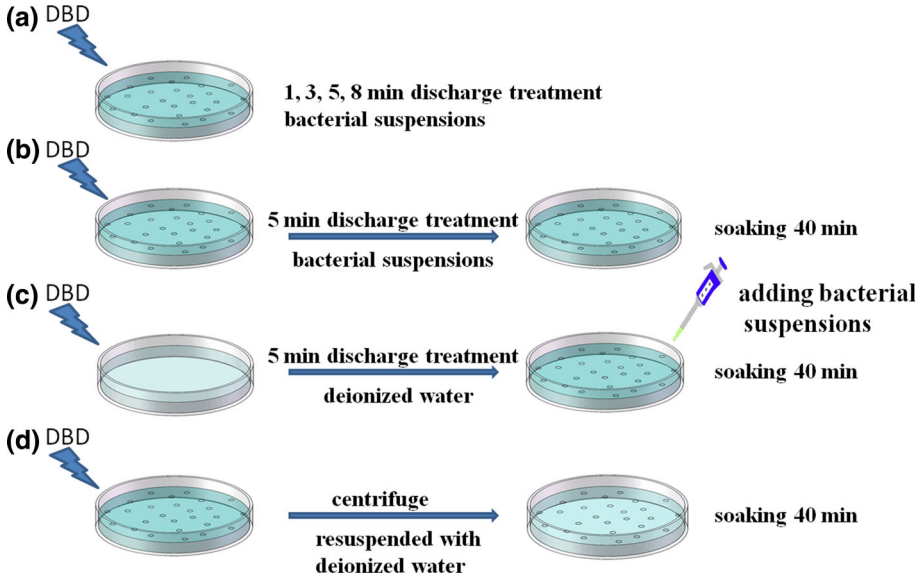

Fig. 2 Samples treated by four different plasma processes

3 ml of deionized water were put on the same dishes and after the discharge treatment for 5 min, 30 µl of the initial bacteria were introduced to the treated deionized water. Both high-concentration and low-concentration bacteria were treated by this way. In the fourth process (d), the bacteria suspension was exposed to the plasma discharge for different periods of time, centrifuged, and resuspended in deionized water with the same volume. After soaking for different periods of time, the surviving bacteria were counted by the dilution method of plate counting to derive the inactivation efficiency.

Result and Discussion

Electrical Properties

To clarify the effects of the different experimental parameters on the plasma properties and bacteria inactivation, experiments are performed with pure helium and helium with water. In the pure helium discharge, two current peaks emerge in the current waveform of the discharge per half-cycle of the applied voltage as shown in Fig. 3a. The current peaks correspond to the step in the Lissajous figure in Fig. 3b. In the case of the helium and water vapor discharge, there are more current peaks in Fig. 3c than Fig. 3a. However, there is no step in the Lissajous figure (d), which is an approximate parallelogram. Water vapor added to helium has an important effect on the discharge leading to the differences between (a) (b) and (c), (d) in Fig. 3. In the pure helium discharge, periodic current peaks appear from the waveform and in addition, the two discharge forms are different. The pure helium discharge is uniform whereas the helium and water vapor one is filamentous with many current peaks. The more current peaks in the discharge, the smaller is the amplitude of each step and therefore, the steps are too small to show in Fig. 3d [33, 34]. In both cases, the discharge power is kept constant at 21 W. Water vapor can absorb a substantial part of the

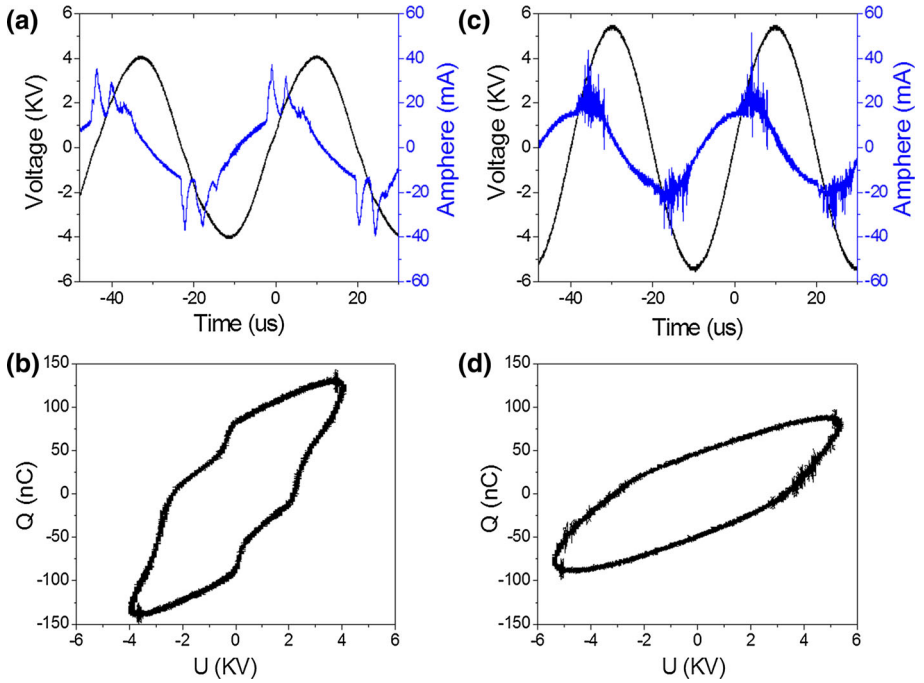

Fig. 3 Characteristics of the helium DBD discharge: **a** waveforms of the applied voltage and measured current, **b** lissajous figure showing the characteristics of helium in the water DBD discharge, **c** waveforms of the applied voltage and measured current, and **d** lissajous figure

electron energy in the discharge and consequently, the applied voltage is larger in Fig. 3c than Fig. 3a. In addition, water vapor is the source of hydroxide radicals, hydrogen radicals, and H_2O^+. Therefore, more reactive species are generated in the case of helium with water discharge. This will be further discussed based on the optical and mass spectra.

Optical Emission Spectroscopy

The optical spectra normalized to a common maximum for easy comparison and the spectra acquired from the two plasmas are shown in Fig. 4 showing a similar range between 200 and 900 nm. In the helium discharge, various nitrogen species peaks are observed in the range of 300 and 400 nm and the main He lines (587.6, 667.8, 706.6, 728.1 nm) and atomic oxygen O lines (777.2, 844.6 nm) are shown in Fig. 4a. In the helium with water discharge, the charged particles react with water molecules to produce more active species. Besides the species mentioned above, OH, H_α and H_β lines appear from Fig. 4b and they help to explain the final generation of reactive species in the liquid. In the discharge region, the dissociated and excited processes are formed by the following reactions [35]:

$$N_2 + e^- \rightarrow 2N + e^- \quad (1)$$

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

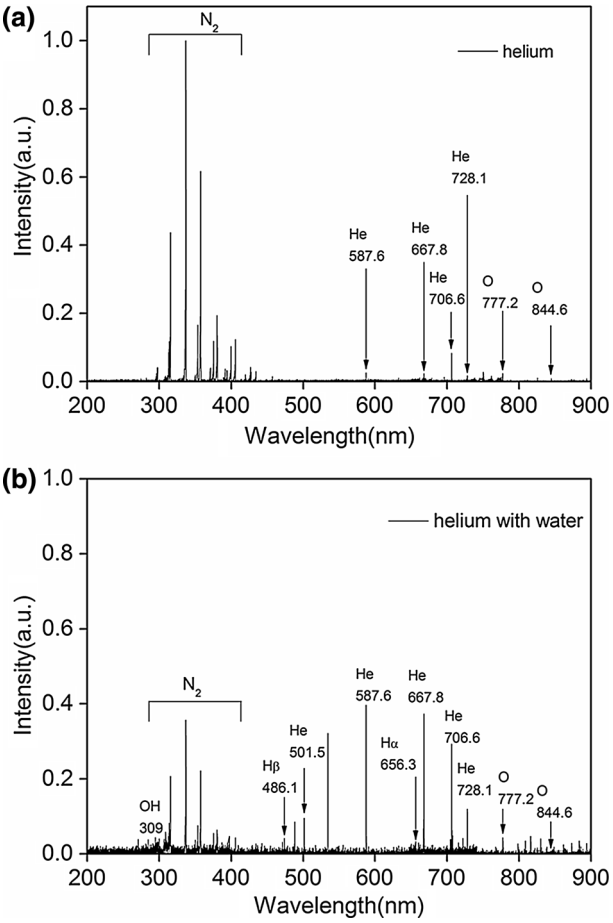

Fig. 4 Optical emission spectrum in the range between 200 and 900 nm: **a** helium discharge and **b** helium with water DBD plasma

$$e^- + He \rightarrow e^- + He* \tag{3}$$

Nitrogen oxides form via the reactions 4, 5 and 6. The produced nitrogen oxides will combine with water to form HNO_3 and HNO_2 to contribute to the decrease of pH:

$$N + O \rightarrow NO \tag{4}$$

$$NO + O \rightarrow NO_2 \tag{5}$$

$$NO + O_3 \rightarrow NO_2 + O_2 \tag{6}$$

In the optical spectra, atomic oxygen combines with O_2 to form O_3 or reacts with H_2O to form hydroxide radicals and hydrogen peroxide in the liquid [35, 36]. O_3 is transferred to water to promote bacteria inactivation:

$$O + O_2 \rightarrow O_3 \quad (7)$$

$$O + H_2O \rightarrow OH\cdot \quad (8)$$

$$O + H_2O \rightarrow H_2O_2 \quad (9)$$

Mass Spectrometry

Time-averaged mass spectra are acquired at 5 mm from the bottom of the quartz glass in the atmospheric-pressure DBD plasma device. Negative ions are generated by dissociative electron attachment to H_2O and positive ions are generated by Penning ionization of metastable helium in addition to dissociative electron impact ionization of water [37]. The positive and negative ions mass spectra are depicted in Fig. 5a and b, respectively. Figure 5a shows about 11 species in the helium DBD plasma with the main species (N^+, O^+, OH^+, H_2O^+,H_3O^+, N_2^+, N_2H^+, NO^+, O_2^+, N_2O^+, NO_2^+) and more than 10 species are observed from the negative spectrum in Fig. 5b with the dominant species being O^-, OH^-, H_3O^-, O_2^-, NO_2^-, O_3^-, $O^-(H_2O)_2$, CO_3^-, HCO_3^-, and NO_3^-.

Figure 5 shows N_2^+, H_2O^+, H_3O^+, NO_2^-, and NO_3^- which help to promote bacteria inactivation. In the gas phase, owing to electron and ion bombardment in the DBD discharge water vapor, reaction 10 occurs to form H_2O^+ and this reaction also occurs in the liquid. Reaction 11 produces hydroxyl radicals and H_3O^+, the H_3O^+ contributing to the decrease of pH value [35]:

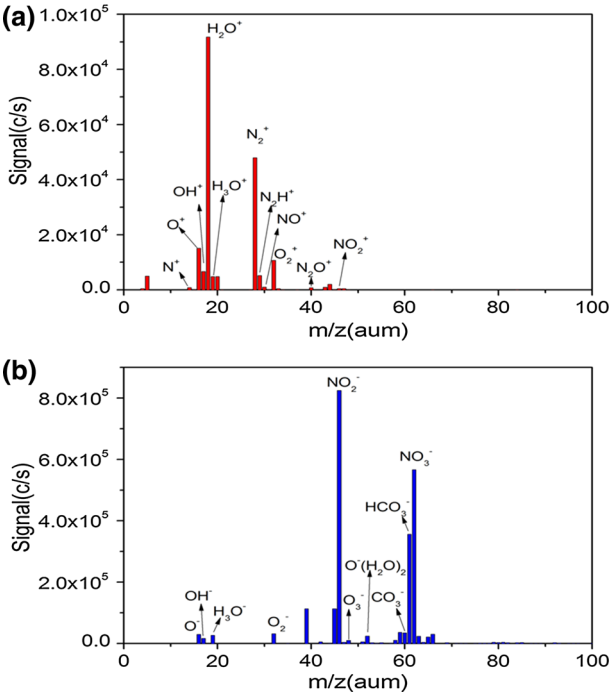

Fig. 5 Mass spectra acquired from the helium DBD plasma. **a** Positive mass spectra and **b** negative mass spectra

$$H_2O + e^- \rightarrow H_2O^+ + 2e^- \quad (10)$$

$$H_2O^+ + H_2O \rightarrow OH\cdot + H_3O^+ \quad (11)$$

$N_2{}^+$ is present in the gas–water DBD discharge and nitrogen oxide is formed by reactions 12 and 13 [38]. At the same time, reactions 5 and 6 occur to contribute to generating HNO_3 and HNO_2 in the liquid:

$$N_2 + e^- \rightarrow N_2^+ + 2e^- \quad (12)$$

$$N_2^+ + H_2O \rightarrow NO + H \quad (13)$$

The positive ions and negative ions impinging in water can generate radicals such as hydroxide radical (OH·) and atomic oxygen (O) or increase the acidity of the surrounding environment, thereby causing subsequent oxidative damage resulting in cell death [39, 40].

Generation of Reactive Species in Liquid

In the gas–liquid discharge process, charged particles are absorbed to produce reactive species including short-lived and long-lived ones. The short-lived reactive species such as hydroxyl radical (OH·) and atomic oxygen (O) have been verified to exist in water. Apart from reactions 8 and 11, OH radicals are generated by the plasma by different reactions [41, 42]. First of all, in the helium plasma discharge, OH radicals produced by metastable He induce water dissociation.

$$He^* + H_2O \rightarrow He + OH(A) + H \quad (14)$$

Secondly, OH radicals are formed by electron bombardment with water causing direct decomposition of water.

$$e^- + H_2O \rightarrow H + OH(A) + e^- \quad (15)$$

Finally, the ground state OH(X) becomes OH(A) when OH(X) has a very large density:

$$OH(X) + e^- \rightarrow OH(A) + e^- \quad (16)$$

In the gas–liquid discharge, electrons, ions, and radicals generated by the DBD plasma react with water producing reactive species. Besides some short-lived reactive ones, long-lived reactive species such as H_2O_2, NO_3-, and O_3 are detected from the treated solution. It has been reported that plasma-treated water has antimicrobial ability. H_2O_2 and O_3 can react with components in the bacteria leading to inactivation and $NO_3{}^-$ and $NO_2{}^-$ acidification the solution [27]. H_2O_2 is generated by the following reaction [43]:

$$OH\cdot + OH\cdot \rightarrow H_2O_2 \quad (17)$$

Atomic oxygen O can form O_2 and O_3 and the production of ozone in liquid is expressed by reactions 2, 18, and 19 [44–46].

$$e^- + H_2O \rightarrow 2H + O + e^- \quad (18)$$

$$O + O_2 + M \rightarrow O_3 + M \quad (19)$$

Long-lived reactive species (NO_3^-, O_3 and H_2O_2) are detected from the solution. Although NO_2^- is also detected, the maximum concentration of about 0.9 mg l^{-1} is very close to the detective limit of 1 mg l^{-1}. As shown in Fig. 6, the concentrations of H_2O_2, NO_3^- and O_3 change with discharge time after the 8-min plasma treatment, the concentration of H_2O_2 increases dramatically from 0 to 126 mg l^{-1}. In comparison, the concentrations of NO_3^- and O_3 go up to 25.2 and 3.9 mg l^{-1}, respectively.

Nitrate and nitrite are formed by reactions 5, 6, 12, 13, 20 and 21 in the plasma discharge [47]. The products reduce the solution pH as shown in Fig. 7.

$$NO_2 + OH\cdot \rightarrow HNO_3 \tag{20}$$

and

$$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3 \tag{21}$$

To determine the concentration of the residual reactive species, the 5-min plasma-treated solution is stored for 40 min and the changes in the concentrations are shown in Fig. 8. The H_2O_2 and O_3 concentrations diminish from 96 to 88 mg l^{-1} and from 3.5 to 3.1 mg l^{-1}, respectively. In contrast, the NO_3^- concentration increases slightly due to the oxidation of NO_2-.

Bacteria Inactivation and Mechanism

The rates of bacterial inactivation by plasmas are not determined merely by the exposure time. There are other factors which have effects on bacteria inactivation efficiency. The initial concentration of the bacteria, bacteria type, conditions of the electrical discharge and liquid exposure methods (direct or indirect) play significant roles in the plasma inactivation kinetics [48]. The inactivation effects of the direct plasma treatment (process a) and indirect treatment (process c soaking for 8 min) for different original bacteria concentrations are shown in Fig. 9. After plasma exposure for 8 min, the high original bacteria concentration shows reduction of 4.4-log CFU ml^{-1}, and the low original bacteria concentration is completely inactivated. After 8-min indirect treatment, the high concentration is reduced by 0.6-log CFU ml^{-1} and the low concentration shows a reduction of 1.8-log CFU ml^{-1}. The results show that bactericidal effects do not vary monotonically with

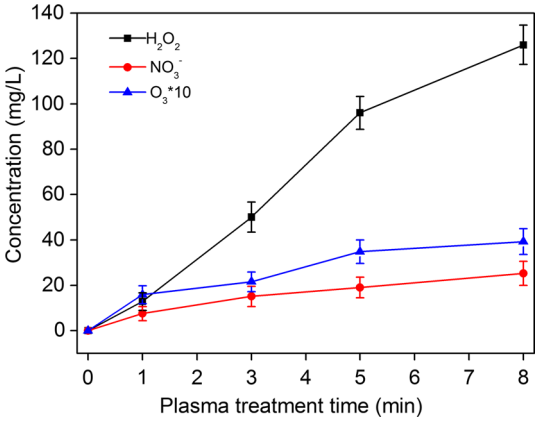

Fig. 6 Concentrations of reactive species as a function of plasma treatment time

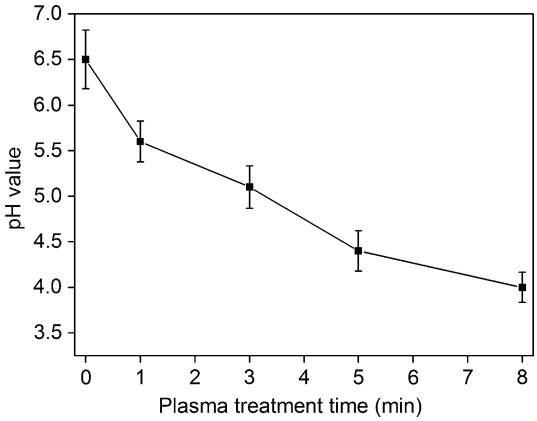

Fig. 7 pH value changes with plasma treatment time

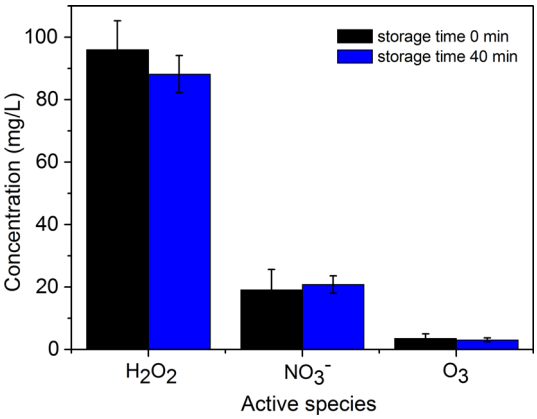

Fig. 8 Concentrations of reactive species after plasma exposure for 5 min and then storage for 40 min

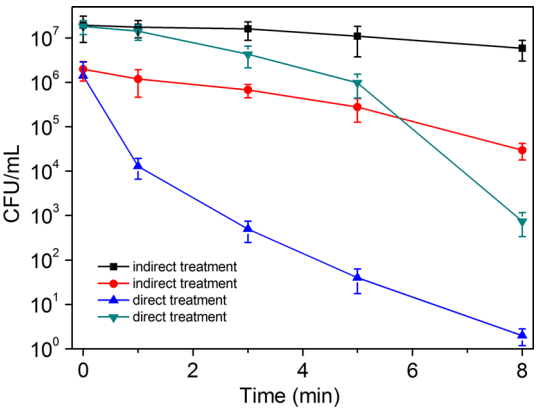

Fig. 9 Bacteria inactivation effect as a result of the direct DBD plasma treatment and indirect treatment for different initial bacteria concentrations

concentrations and lower concentration can accelerate the bacterial process [49]. The results demonstrate the difference between the direct and indirect treatment and the former shows higher bacterial efficiency. The direct treatment shows the synergistic effects of

long-lived and short-lived reactive species as well as physical effects, whereas the indirect treatment reflects the effects of long-lived reactive species on bacteria inactivation.

In the gas–liquid discharge, complex physical and chemical processes cause bacteria inactivation. The physical effects include heat, UV emission, and electric field. In the discharge, some of the power dissipates in the liquid raising the water temperature. Nonetheless, the maximum temperature of the treated water is still below 35 °C which cannot lead to bacterial inactivation. The UV emission shown in Fig. 4 is also too weak (from 200 to 300 nm) to cause bacteria inactivation [23, 50, 51]. In fact, water absorbs some of the UV which is thus too weak to inactivate bacteria.

Ma et al. have shown that the electric field alone makes a minor contribution to liquid-phase bacteria inactivation in the DBD plasma [4]. Energetic particles such as active He, electrons, and ions are absorbed by water molecules to create reactive species in the liquid as mentioned above. Energetic particles are involved in chemical reactions in the liquid instead of imposing direct effects on the bacteria in the liquid-phase [40, 52]. Besides these factors, reactive species associated with chemical effects are investigated. According to Figs. 4 and 5, short-lived reactive species such as O and OH· are generated by the DBD plasma discharge. Atomic oxygen and hydroxyl radicals play a significant role in bacteria inactivation due to the interaction between OH· and O and bacteria [50, 53, 54]. The hydroxyl radical possesses an oxidation potential of 2.8 V and atomic oxygen has an oxidation potential of 2.4 V. Both are very reactive and can disrupt cellular functions. They even produce reactive species which penetrate the bacteria damaging the cellular structure [55, 56]. Hence, hydroxyl radicals and atomic oxygen can cause oxidative damage detrimental to bacteria [57, 58].

Long-lived reactive species such as hydrogen peroxide, nitrate, nitrite, and ozone have been demonstrated to induce inactivation [59, 60]. Here, these long-lived reactive species are produced in the liquid. To differentiate the bacterial effects of the long-lived reactive species and short-live reactive species, indirect treatment (process c) is carried out. Figure 9 shows the different contributions of long-lived and short-lived reactive species in the various treatment processes on bacteria inactivation. The indirect treatment reflects the inactivation ability of long-lived reactive species whereas the direct treatment shows the inactivation ability of both the long-lived and short-lived reactive species. In the short time treatment (soaking for less than 8 min), the long-lived reactive species with the maximum concentration in the liquid play a small role in bacteria inactivation. However, after the direct treatment for 8 min, the inactivation efficiency with a high original bacteria concentration shows more than 4-log CFU ml^{-1} reduction. It means that more than 99.99% of the initial population of the bacteria is inactive and the low-concentration bacteria are completely inactivated. The results suggest that short-lived reactive species such as OH and oxygen atoms play an important role in bacteria inactivation during the plasma treatment but that of the long-lived reactive species is minor for a short time.

The different treatment processes can utilize long-lived reactive species alone or the synergistic effects of free radicals and long-lived species to achieve different bacterial efficiency. Here, more experiments are performed: (1) Soaking for 40 min after the direct discharge treatment for 5 min (process b) and (2) Soaking for 40 min together with 5-min plasma treatment in deionized water with different concentrations (process c). The bacteria inactivation effects in Fig. 10 show that process c (rectangle) reduces the bacteria by about 3-log CFU ml^{-1} and process b for the same initial concentrations (triangles) leads to reduction of 7-log CFU ml^{-1}. In process b, after the direct treatment for 5 min, the concentration of bacteria drops initially and the residual effect on bacteria inactivation is also pronounced. The direct discharge treatment inactivates bacteria and forms reactive

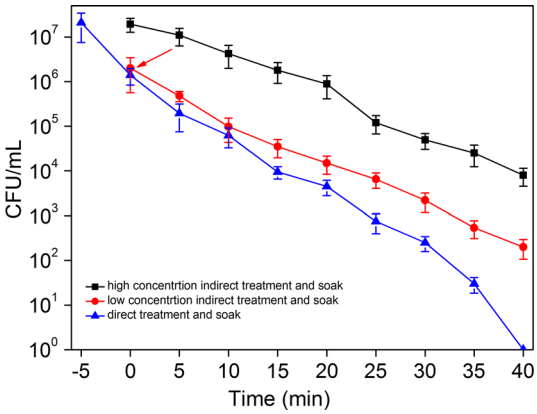

Fig. 10 Bacteria inactivation effect after direct DBD plasma direct treatment and indirect treatment for 5 min followed by storage for 40 min

oxidative species such as hydroxyl radicals and hydrogen peroxide causing initial damage to the bacteria to promote bacteria inactivation. As indicated by the arrow, from this point, the bacteria concentration in processes b and c is the same, but the inactivation efficiency is quite different. Process b shows a higher inactivation rate than process c. It is because the direct discharge treatment causes initial damage to the bacteria resulting in partial bacteria inactivation and accelerates inactivation by the long-lived reactive species. The results suggest that the initial damage caused by the plasma discharge contributes to inactivation [61].

To verify the effects of initial damage in bacteria inactivation, more experiments are performed. The bacteria suspensions are directly exposed to the DBD plasma for 1, 3, 5, or 8 min and then the suspensions are centrifuged and resuspended in deionized water prior to storage for 40 min (process d) to investigate the effects of the initial damage. To observe the effects of the initial damage, control samples which do not undergo the plasma treatment are prepared. Figure 11 shows that the control sample is hardly inactivated and the residual bacteria display increasing inactivation efficiency with time in deionized water. Even short plasma exposure for 1 and 3 min gives rise to bacteria damage and subsequent inactivation as manifested by that 9.3 and 37.2% of the bacteria are disinfected. For longer plasma treatment time such as 5 and 8 min, 81.8 and 86.7% of the resuspended

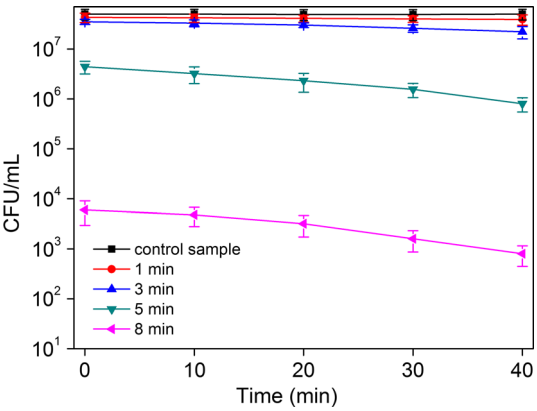

Fig. 11 Effects of initial damage on residual bacteria for different treatment time

bacteria are deactivated, demonstrating that even mild damage to the bacteria may cause bacteria inactivation.

The effects of different treatment processes on bacteria inactivation are quite different. Among the three indirect treatment processes, a short storage time leads to low bactericidal efficiency and direct soaking requires a long time to accomplish efficient bacteria inactivation, implying that the long-lived reactive species such as H_2O_2, NO_3^-, and O_3 alone impose residual destructive effects on the bacteria but a longer time is needed for better inactivation effects. Compared to the other two processes, direct plasma treatment with soaking exhibits the best inactivation efficiency. Not only the short-lived and long-lived reactive species produced by the plasma cause initial damage to the bacteria, but also the long-lived reactive species produced by the discharge have continuous residual effects on bacteria inactivation. In the presence of liquid products created by the plasma, better bactericidal efficiency and higher energy efficiency are achieved.

Conclusion

The atmospheric helium dielectric barrier discharge (DBD) is applied to treat *S. aureus* to investigate the inactivation mechanism and optimize the bacteria inactivation efficiency. Different treatment methods are adopted to analyze the gas–liquid plasma characteristics, generation of reactive species, physical and chemical processes, as well as formation of liquid products. Combination of different treatment processes and different initial bacteria concentration cause various effects on bacteria inactivation. The short-lived reactive species are efficient in inactivating bacteria and the long-lived ones impose long-term residual effects on bacteria inactivation. The results show that attention must be paid to initial damage caused by direct plasma exposure in addition to the residual effects. Optimization of the experimental parameters such as the discharge time and storage time can produce the desirable effects.

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