

Review

# Plasma-Activated Water: Physicochemical Properties, Generation Techniques, and Applications

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**Abstract:** Plasma-activated water (PAW) is water that has been treated with atmospheric pressure plasma. Due to the presence of reactive oxygen and nitrogen species (RONS), PAW can be used in various applications such as (1) surface disinfection and food decontamination, (2) enhancement in seed germination, and (3) enhancement in surface cooling in the nucleate boiling regime. Briefly, for surface disinfection, the reactive species in PAW can induce oxidative stress on microbes; for enhancement of seed germination, the reactive species in PAW can trigger seed germination and provide nutrients; for enhancement in surface cooling, the reactive species cause a reduction in the surface tension of PAW, facilitating the phase-change heat transfer and, quite unexpectedly, minimizing the surface oxidation. Here, we review the physicochemical properties of PAW, the three commonly used techniques (plasma jet, dielectric barrier discharge, and corona discharge) for generating atmospheric pressure plasma, and the use of PAW for the above three applications. In particular, we review the recent development of the miniaturization of the plasma generator integrated with an acoustic neutralizer to produce plasma-activated aerosols, elimination of the need for storage, and the interesting physicochemical properties of PAW that lead to cooling enhancement.

**Keywords:** plasma-activated water (PAW); plasma jet; dielectric barrier discharge; corona discharge; disinfection; germination; cooling



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## 1. Introduction

Plasma-activated water (PAW) is water that has been treated by a stream of ionized gas (plasma), which is rich in reactive species [1]. In recent years, many studies have shown that PAW can provide effective inactivation of pathogenic microbes [2,3]. Additionally, PAW also can enhance seed germination [4] and surface cooling (in the nucleate boiling regime) [5,6], as well as the anaerobic treatment of palm oil mill effluent [7]. The effectiveness of PAW in these applications is strongly dependent on the concentration of reactive species, which can be categorized into two major groups: reactive oxygen species (ROS) and reactive nitrogen species (RNS).

For application in disinfection, the ROS generated in PAW such as hydrogen peroxide, hydroxyl radical, and ozone are strong oxidizing agents and are capable of inducing oxidative stress on the microbial cell membranes. For instance, the structure and chemical bonds of the cell membrane protein of *Staphylococcus aureus* can be altered after being exposed to PAW [3]. These ROS in PAW are also capable of promoting lipid oxidation in the cell membrane [8,9]. The ROS can destroy the intramolecular bonds of peptidoglycan by removing a hydrogen atom from the peptide bond from the peptidoglycan layer that is present outside the plasma membrane of most bacteria, which can lead to cell wall breakdown, stoppage of metabolic activities, cell damage, and death [3,10–12]. For application in seed germination, on the other hand, ROS plays a role in regulating phytohormones levels, thereby triggering cell metabolism process [13]. Regulating the concentration of ROS is crucial, because excessive concentrations can induce high oxidative stress that inhibits the

germination process [4,14,15]. Similarly, RNS serve as a source of nutrition for plants and regulate cellular redox status, facilitating cell development [16].

The presence of reactive oxygen and nitrogen species (RONS) in PAW can change the physical properties of the solutions. For instance, by increasing the electrical conductivity of the PAW, surface tension can be reduced significantly, leading to higher evaporate rates (for phase-change heat transfer) and thus heat removal rates [5,6]. Here, we first discuss the physiochemical properties of the PAW (Section 2), the common techniques to produce PAW (Section 3), the applications of PAW (Section 4), and finally, the recent efforts to develop miniaturized plasma generator for directly plasma-activated aerosols on contaminated surfaces (Section 5).

## 2. Physicochemical Properties of Plasma-Activated Water

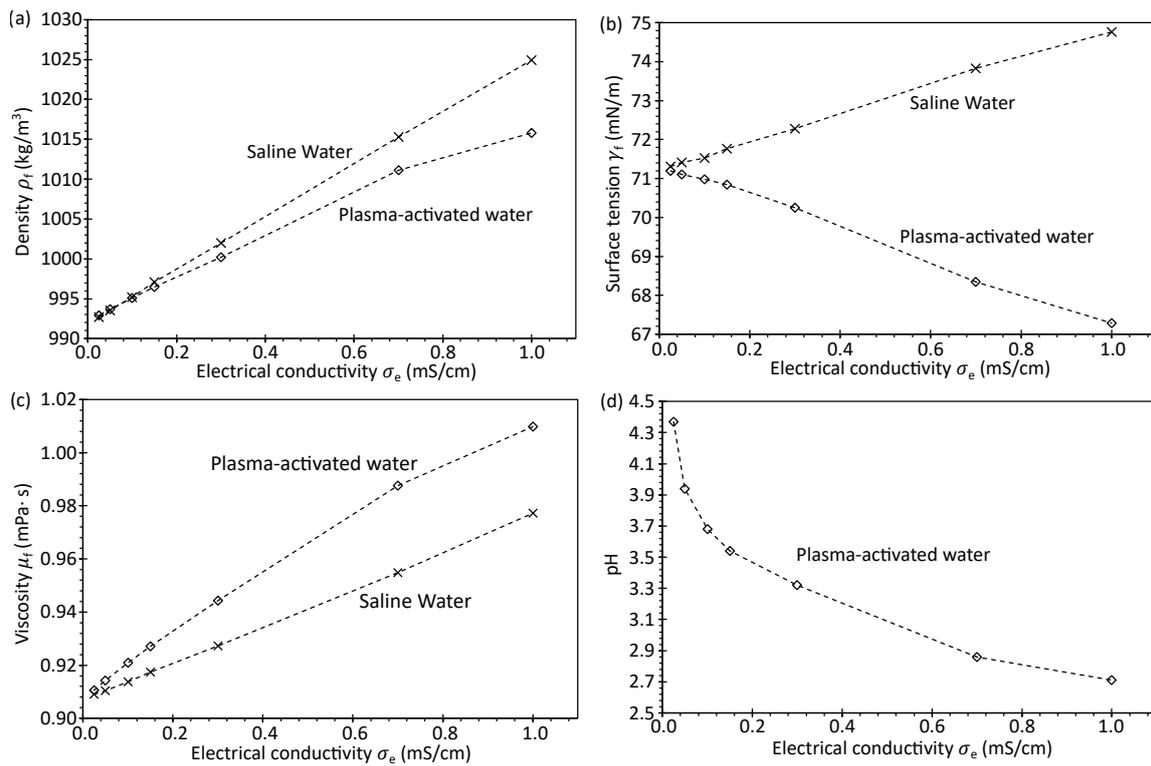
As the plasma comes into contact with water, various RONS are produced at the air–liquid interface. The generated primary reactive species then react with the water molecules and each other to form various secondary species that are crucial to the applications [17]. The generated active species can be separated into long-lifetime species such as hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), ozone ( $\text{O}_3$ ), nitrite ( $\text{NO}_2^-$ ) and nitrate ( $\text{NO}_3^-$ ), which typically have a half-life of minutes to days [18,19] and short-lifetime species such as hydroxyl radicals (OH), nitric oxide (NO), and peroxyxynitrite ( $\text{ONOO}^-$ ), which typically have a half-life ranging from one nanosecond to several seconds that quickly react to create stable species [20,21]; this is the motivation to develop in situ application of plasma-activated water/aerosols (see Section 5). Here, we discuss the physical properties (Section 2.1), ROS (Section 2.2), and RNS (Section 2.3) in PAW, as well as common analytical methods for reactive species measurement (Section 2.4).

### 2.1. Physical Properties

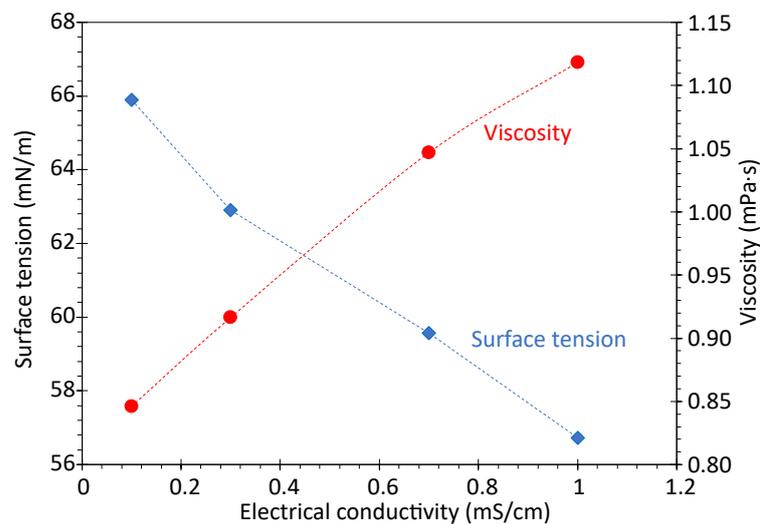
**Density**—the density of PAW is directly proportional to the concentration of ions, as shown in Figure 1a. During the plasma treatment, various free-moving charged species and ions diffuse into the water, resulting in higher concentrations of these ions and thus an increase in density [5,6].

**Surface tension**—the surface tension of PAW decreases with the increase in electrical conductivity, as shown in Figures 1b and 2. The reduction in surface tension could be attributed to an increase in  $\text{NO}_3^-$  ions in PAW, which can interfere with the hydrogen bond of the water molecule when the  $\text{NO}_3^-$  ions are attracted to the air–liquid interface [22,23].

**pH**—the pH of PAW decreases with the increase in treatment duration, as shown in Figure 1d [24–26]. However, under an extended treatment duration, the rate of reduction in pH is reduced and subsequently reaches a steady-state where it remains constant. Despite its acidity and low pH, PAW is considered safe for application on the skin as a disinfectant. It is neither toxic nor corrosive [27]. Studies have demonstrated that when PAW is applied to mice, it can facilitate skin wound healing without causing any damage to the tissues [28,29]. Furthermore, an in vivo study conducted on mice with pancreatic cancer cells showed that PAW is non-toxic and safe for use in animals [30]. When the pH of PAW is relatively neutral, the concentration of reactive species such as  $\text{H}_2\text{O}_2$ ,  $\text{NO}_2^-$ ,  $\text{NO}_3^-$ , and  $\text{ONOO}^-$  can remain stable over time. However, at lower pH values ( $\text{pH} < 3.5$ ), the reactive species become unstable and undergo active reactions with each other, leading to an unstable concentration of reactive species [31,32]. Nonetheless, a recent study demonstrated a hybrid conservation and restoration method for PAW through low-temperature storage and pH adjustment [33]; this method can efficiently preserve the bactericidal effect of PAW for up to 10 days.



**Figure 1.** By exposing deionized water to atmospheric pressure plasma (to produce PAW), due to the dissolved and the subsequent formations of reactive species in the water, the physical properties such as (a) density, (b) surface tension, (c) viscosity, and (d) pH can be altered significantly. As can be seen here, the higher the concentration of these reactive species, the higher the electrical conductivity  $\sigma_e$  of the PAW. Note that the PAW was produced via the corona discharged plasma. From [34]. Copyright 2023 Author(s), licensed under a Creative Commons Attribution (CC BY-NC-ND 4.0) License.



**Figure 2.** PAW produced using plasma jet (with compressed air as feeding gas) has similar characteristics to those produced using the corona discharge plasma (see Figure 1), i.e., by increasing the electrical conductivity of the solutions, the surface tension is reduced, whereas the viscosity is increased.

**Electrical conductivity**—As PAW consists of various free-moving charged species and ions due to the diffusion of reactive species into the water while being treated by plasma, the conductivity is increased. Studies have shown that the conductivity of PAW can increase

to the range of 100–500 mS/cm [25,35,36]. The electrical conductivity of PAW is one of the important indicators of the overall concentration of the reactive species and ions.

**Oxidation–reduction potential**—PAW has higher oxidation–reduction potentials. For instance, hydrogen peroxide  $\text{H}_2\text{O}_2$  is one of the important species that leads to higher oxidation–reduction potentials due to its ability to act as an oxidant or as a reductant [37]. Similarly, ROS such as hydroxyl radical OH and ozone  $\text{O}_3$ , as well as RNS such as nitrates  $\text{NO}_3^-$ , nitrites  $\text{NO}_2^-$ , and peroxyxynitrite  $\text{ONOO}^-$  also contribute to the high oxidation–reduction potential [38].

## 2.2. Reactive Oxygen Species (ROS)

**Hydroxyl radical (OH)** is the precursor of hydrogen peroxide found in PAW and is a strong oxidizing agent [39]. It has a short lifetime of approximately 200  $\mu\text{s}$  in the gas phase and only several nanoseconds in the liquid phase due to its high reactivity [40]. OH has a vital role in the formation of various reactive species especially in the gas phase (prior to their diffusion into the liquids). The concentration of the reactive species in the PAW for antimicrobial application is closely related to the concentration of the OH [41]. Due to its short lifetime, the OH found in PAW is usually from secondary reactions such as the breakdown of hydrogen peroxide and the reaction of ozone with hydrogen peroxide or hydroperoxyl radical [42].

**Hydrogen peroxide ( $\text{H}_2\text{O}_2$ )** is the most common long-lifetime species found in PAW and has an important role in antimicrobial and seed germination [4,43,44].  $\text{H}_2\text{O}_2$  is mainly generated in PAW through two pathways: (1) OH radicals generated in the gas phase combine into  $\text{H}_2\text{O}_2$  and then diffuse directly into the liquid phase and (2) OH radicals generated in the gas phase diffuse into the liquid phase and then combine to form  $\text{H}_2\text{O}_2$  [41,45].  $\text{H}_2\text{O}_2$  is capable of freely diffusing through the cell membrane without disrupting it. Hence, hydrogen peroxides that cooperate with it are able to damage and increase the permeability of the cell membrane, leading to the ease of extracellular reactive species in the PAW to invade the targeted cell [46].

**Ozone ( $\text{O}_3$ )** in an aqueous solution is a strong antimicrobial oxidizing agent that possesses one of the highest oxidation–reduction potential values among typical oxidants such as permanganate,  $\text{H}_2\text{O}_2$ , chlorine, and chlorine dioxide [47]. It is mainly generated in PAW through two pathways: (1)  $\text{O}_3$  generated in the gas phase diffuses directly into the liquid phase and (2)  $\text{O}_3$  generated in the liquid phase by plasma discharge in bubbles containing oxygen [37]. Nevertheless, a previous study indicated that the  $\text{O}_3$  concentration may be negligible in PAW due to its aggressive reactions with nitric oxide and nitrogen dioxide in the gas phase, and, also with nitrite in the liquid phase [18].

**Superoxide ( $\text{O}_2^-$ )** is yet another important ROS that is generated when water is treated with atmospheric pressure plasma. It is mainly produced from (1) the highly energetic electrons ejected by the plasma reacting with oxygen molecules, (2) OH reacting with  $\text{O}_3$ , and (3) the secondary reaction of peroxyxynitrite into  $\text{O}_2^-$  and nitric oxide. A previous study showed that  $\text{O}_2^-$  is essential for bacteria inactivation because the critical pH value for antimicrobial effects is strongly related to the acid dissociation constant between the  $\text{O}_2^-$  and hydroperoxy radicals, both of which interact and inactivate bacterial cells [48].

## 2.3. Reactive Nitrogen Species (RNS)

**Nitrites ( $\text{NO}_2^-$ )** and **nitrates ( $\text{NO}_3^-$ )** are both long lifetime species, generated as secondary products in PAW. In a low pH environment, nitrite easily degrades and protonates into  $\text{H}_2\text{O}_2$ , nitric oxide and nitrogen dioxide that are considered to be very toxic to cells [49,50].  $\text{NO}_2^-$  are produced from nitrogen oxides ( $\text{NO}_x$ ) that are generated through the reaction between nitrogen and oxygen in the gas phase during the plasma treatment [51]. When  $\text{NO}_2^-$  interact with an oxidizing agents such as  $\text{H}_2\text{O}_2$  and ozone, it can convert to  $\text{NO}_3^-$  [52]. Thus, the formation of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in PAW is accompanied by ROS, such as  $\text{H}_2\text{O}_2$ . Furthermore, the production of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in PAW is affected by the treatment conditions and type of plasma generator used [24,53].

**Nitric oxide** (NO) is a signaling molecule and can be found ubiquitously in various organisms. It has the ability to inhibit bacteria, induce apoptosis in cancer cells, and help in wound healing [54]. NO in the PAW can be formed by the dissolution of NO from the gas phase into the liquid phase or by secondary reactions in the PAW [55]. Nonetheless, due to the low solubility of NO in water and its low to moderate gas phase density, the NO found in PAW is mainly from the secondary reaction between nitrogen dioxide with atomic oxygen or  $O_3$  [56]. Recent studies indicate that NO has a short lifetime and converts into  $NO_2^-$  and  $NO_3^-$  quickly in PAW [46,57].

**Peroxynitrite** ( $ONOO^-$ ) is a strong oxidizing and nitrating agent that has an important role in disease regulation and can help combat neuro-degeneration, inflammation, diabetes, and other pathologies [58]. The formation pathways of  $ONOO^-$  are mainly from the reaction of (1) NO with  $H_2O_2$  or  $O_2^-$ , and (2)  $NO_2^-$  with  $H_2O_2$  or hydroperoxyl radicals [59,60]. As both  $NO_2^-$  and  $H_2O_2$  have a strong synergistic effect in the inactivation of microorganisms and they react to form  $ONOO^-$  in an acidic environment,  $ONOO^-$  can potentially be a crucial species in the antimicrobial properties in PAW [52].

#### 2.4. Methods to Measure Reactive Species

Two commonly used analytical techniques to measure the reactive species in PAW are electron spin resonance and UV absorption spectroscopy.

**Electron spin resonance** (ESR) can be used to detect and quantify free radicals in PAW. During measurement, the sample is subjected to horizontal magnetic and vertical microwave radiation [61]. The free radicals within the sample are excited when the frequency of the microwaves meets the resonance condition; their energy fluctuation is proportional to the magnetic field strength. To detect short-lifetime species, a spin trap reagent reacts with free radicals and forms stable spin-trapped adducts, which can be analyzed using ESR spectroscopy. Different spin-trapped reagents are used depending on the specific reactive species of interest, and the resulting spin-trapped adducts exhibit distinct ESR spectra, enabling their identification and quantification [53,62,63].

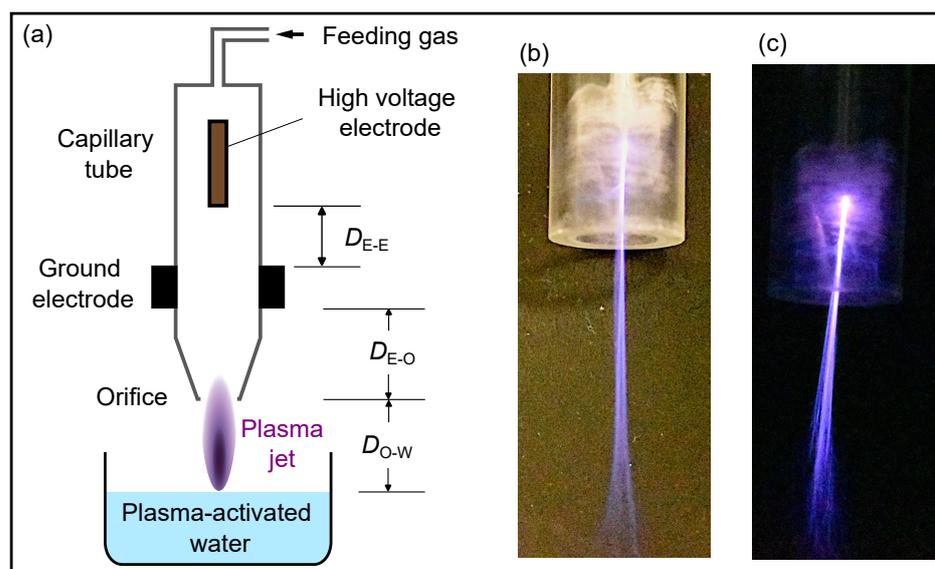
**UV absorption spectroscopy** (UV-Vis) is another technique used to quantify the reactive species by measuring the absorption of ultraviolet (UV) and visible light by the PAW. A spectrophotometer is used to emit a broad spectrum of light that includes UV and visible wavelengths. As the light passes through the sample, the reactive species absorb the light based on their characteristic absorption patterns at specific wavelengths, and the detector measures the amount of light absorbed by the sample at different wavelengths. Each reactive species has unique absorption peaks and bands at specific wavelengths. Chemical probes are often used in conjunction with UV-Vis spectroscopy to enhance the detection of specific reactive species. These probes react with the reactive species to form colorimetric or fluorogenic products that can be easily detected. Different chemical probes are used depending on the targeted reactive species [53,62,63].

### 3. Generation Techniques: Atmospheric Pressure Plasma for the Production of Plasma-Activated Water

Generally, for the production of PAW, there are three commonly use techniques to generate atmospheric pressure plasma: (1) plasma jet (Section 3.1), (2) dielectric barrier discharge (Section 3.2), and (3) corona discharge (Section 3.3).

#### 3.1. Plasma Jet

A stable and uniform plasma jet is generated when a time-varying high voltage is applied to the electrode, and the amplitude is sufficient to ionize the compressed gas that flows within the capillary tube [64], as illustrated in Figure 3. In general, the physico-chemical properties of PAW generated from the plasma jet are relied on both the physical parameters (the geometry of the setup) and the operating parameters (types of feeding gases and their flowrate, volume of treated water, electrical power, and treatment duration).



**Figure 3.** (a) Sketch illustrates the common setup to generate plasma jet for the production of PAW. Briefly, the setup consists of a supply of feeding gas, two electrodes, and a capillary tube. The electrode inside the capillary tube is connected to a high-voltage power supply, and the electrode outside the capillary tube is connected to the ground terminal. When a high voltage is applied across the electrodes, plasma is generated. The strong electric field on the surface of the dielectric tube (capillary tube) can break down the gas molecules and accelerate electrons. Images (b,c) show the typical plasma jet with nitrogen as feeding gas.

To generate a plasma jet for the efficient production of PAW, the important setup parameters are (1) the distance between the high voltage and ground electrodes  $D_{E-E}$ , (2) the distance between the ground electrode and the orifice  $D_{E-O}$ , and (3) the distance between the orifice to the liquid–air interface  $D_{O-W}$ . For the first setup parameter, the concentration of  $H_2O_2$  concentration in PAW can be controlled by adjusting the distance between the high-voltage and ground electrodes  $D_{E-E}$  [65]. This can be attributed to the increase in charge accumulation on the inner surface of the capillary tube as the distance between the electrodes is increased, facilitating the ionization process. Furthermore, the discharge volume is increased by increasing  $D_{E-E}$ , leading to higher electron densities and temperatures [66], and thus increase the rate of water evaporation (at the liquid–air interface). This allows the plasma to chemically react with the evaporated water molecules to form  $H_2O_2$  ions [67–69].

For the second setup parameter, increasing the distance between the ground electrode and the orifice  $D_{E-O}$  causes electrons to travel a longer distance before leaving the orifice, delaying electron propagation and reducing the electron density. Consequently, it interrupts the interactions of short-lifetime species and leads to lower concentrations of reactive species in the PAW [65,70]. For the third setup parameter, the increase in distance between the orifice and the liquid–air interface  $D_{O-W}$  decreases the electrical conductivity, oxidation–reduction potential, and reactive species concentrations in PAW [69]. Particularly, with the increase in  $D_{O-W}$ , the production rate of  $H_2O_2$  in the PAW is reduced; however, the production of nitrogen species is not affected [67]. This can be attributed to the decrease in the electron temperature and the increase in the electron density, i.e., lower electron temperatures reduce the evaporation of water molecules and hence decrease the formation rate of  $H_2O_2$ , whereas higher electron densities increase the collision rate between heavy neutrals (nitrogen molecules), exciting them into first metastable states, which leads to the formation of nitrogen species [67,71].

The operating parameters can also have significant effects on the concentration of reactive species in PAW. These operating parameters are the type of feeding gases and their flowrate, volume of treated water, electrical power, and treatment duration. The feeding

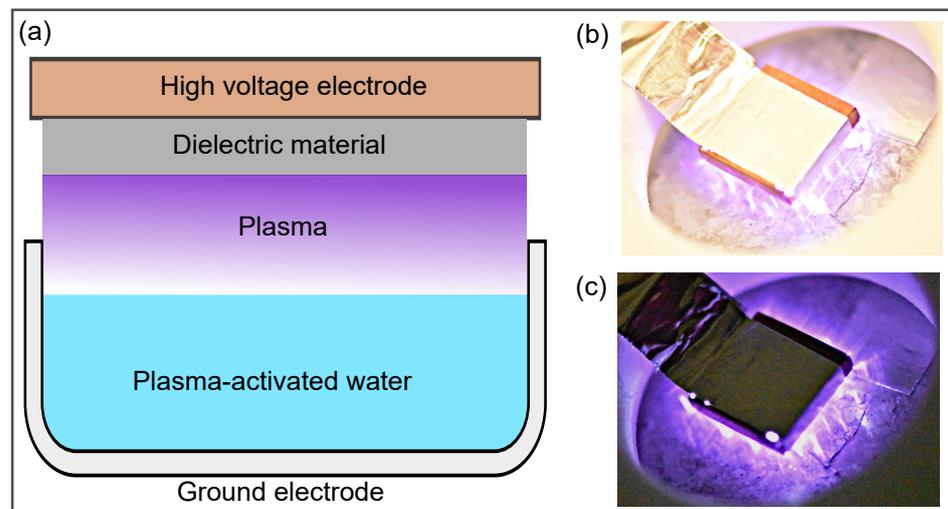
gas refers to the gas that sustains the plasma, while the surrounding gas is the ambient gas outside the orifice. The most commonly used feeding gases are argon, helium, and air. The plasma jet generated with helium has a lower discharge current, longer plasma length, and higher levels of O and  $N_2^+$  ions, whereas the plasma jet generated with argon has a shorter jet length, higher discharge current, and higher levels of OH and nitrogen ions. Helium metastable energy is capable of ionizing and exciting nitrogen into  $N_2^+$  and OH, while the energy of argon is insufficient to ionize nitrogen [72]. PAW produced using plasma jet with argon has the lowest electrical conductivity and  $NO_3^-$  concentration, but the highest  $H_2O_2$  concentration and pH values. Conversely, PAW produced using plasma jet with air has the highest electrical conductivity and  $NO_3^-$  concentration, but the lowest  $H_2O_2$  concentration and pH value. This is because plasma jet with argon can generate higher OH and nitrogen emissions, whereas plasma jet with air can generate high nitrogen but low OH emissions [73]. Mixing different feeding gases can further alter the reactive species concentrations. For instance, using the mixture of nitrogen and argon gases, the concentration of  $NO_2^-$  and  $NO_3^-$  ions can be increased, but the concentration of  $H_2O_2$  is decreased in PAW. On the other hand, using the mixture of oxygen and argon gases, both the concentration of  $H_2O_2$  and  $NO_3^-$  ions can be increased. The oxygen feeding gas increases the oxidizing substances like O and  $O_3$ , which oxidize the gaseous NO and  $NO_2^-$  ions, whereas the nitrogen feeding gas has a higher electron density that increases the interaction of active electron, hydrogen atoms, and hydroxyl radical to form RNS [74–77].

Another important operating parameter is the flowrate of feeding gas. For instance, for the plasma jet with argon, higher flowrates of argon gas can produce higher concentrations of  $H_2O_2$  in PAW [65]. This can be attributed to the residual humidity in commercial argon gas, which increases with the flow rate, leading to an increase in the level of water molecules in the discharge region, where they can dissociate into OH. Furthermore, higher flowrates can increase the transmission of electrical energy to gas molecules and the transportation of OH to the target solution, reducing the hydroxyl quenching reactions with the ambient air. Nonetheless, excessive flowrate of feeding gas can decrease the electron excitation temperature and  $H_2O_2$  concentration [78,79]. The concentration of reactive species in PAW is also influenced by the volume of treated water. For instance, the concentration of  $H_2O_2$  in PAW treated by kHz plasma jet increases linearly regardless of the treated volume [76]. However, concentrations of  $NO_2^-$  and  $NO_3^-$  reach saturation at the smaller volume (25 mL), but not observed in larger treated volumes (125 mL and 200 mL), which was attributed to the saturation effect likely due to recombination processes involving the high concentration of  $H_2O_2$  [76].

The electrical power of plasma jet is another important operating parameter. Higher electrical powers can increase the electrical conductivity and oxidation–reduction potential, but decrease the pH value of the PAW. This is because higher electrical powers can increase the electric field within the plasma jet, inducing a stronger intensity, longer plasma length and higher electron density [72,80–82]. In addition, the physicochemical properties of PAW generated by plasma jet also greatly influence by treatment duration. Studies reported that the electrical conductivity, oxidation–reduction potential and the concentration of reactive species in PAW increase linearly with the increase in treatment duration with a range of 0 to 20 min of treatment duration [69,74,83,84]. Specifically, the conductivity of PAW reaches 1500  $\mu S/cm$  and the concentration of  $H_2O_2$  and formation of  $NO_2^-$  and  $NO_3^-$  reaches 70  $\mu M$  and 15 mM, respectively, after 15 min of plasma jet treatment duration [69]. However, this trend is no longer obtainable if the treatment duration extended beyond the optimum duration. Zhou et al., demonstrated that the concentration of ROS increases linearly regardless the treatment duration, whereas the concentration of RNS reaches saturation after 20 min of treatment duration [52]. Similar to the volume of treated liquid, this saturation effect is likely due to recombination processes involving the high concentration of  $H_2O_2$  present in the smaller volume.

### 3.2. Dielectric Barrier Discharge

Unlike the plasma jet, dielectric barrier discharge can generate atmospheric pressure plasma directly in ambient air without the need to supply feeding gas, as illustrated in Figure 4. Thus, the dielectric barrier discharge is scalable for the mass production of PAW [85–88]. For dielectric barrier discharge, the important setup parameters that have significant effects on the concentration of reactive species in PAW are (1) material for the electrode, (2) material for the dielectric layer/substrate, and (3) distance between the electrodes, and (4) thickness of the dielectric layer/substrate. For the first setup parameter, the electrode's material, when comparing silver, stainless steel, and brass electrodes, due to the strong oxidation of silver, dielectric barrier discharge with silver electrodes can lead to higher concentrations of reactive species emission and  $\text{NO}_3^-$  concentration in PAW, but lower concentrations of  $\text{O}_3$  [89].



**Figure 4.** (a) Sketch illustrates the common setup to generate dielectric barrier discharge for the production of PAW. Briefly, the setup consists of electrodes and dielectric material. When a high voltage is applied across the electrodes, the dielectric material (between the electrodes) accumulates charges on its surface, creating an electric field that counteracts the external electric field and limits current flow, thus preventing sparks or arcs. The high electric field between the electrodes breaks down gas molecules, forming plasma. Images (b,c) show the typical dielectric barrier discharge where glass substrate was used as the dielectric barrier.

For the second setup parameter on the dielectric layer/substrate's material, alumina, which has a high thermal conductivity and surface roughness, can promote the generation of filaments and facilitate heat dissipation. Quartz, on the other hand, can sustain a high electrical potential applied between the electrodes, leading to higher gas ionization rates, thus increasing the concentration of reactive species in PAW [90]. For the third setup parameter on the distance between the electrodes, due to the accumulation of electrons near the dielectric barrier, decreasing the distance can lead to higher concentrations of reactive species [91], giving rise to the high microbial inactivation [92–94]. For the fourth setup parameter, the thickness of the dielectric layer/substrate, thicker dielectric layer/substrates can sustain higher electrical potential between the electrodes, leading to a higher density of micro-discharges with a uniform spatial distribution in the entire discharge region [90].

The important operating parameters that have significant effects on the concentration of reactive species in PAW are: (1) electrical power, (2) treatment duration, and (3) surrounding gas. For electrical power, the important factors are the applied frequency, voltage, current, and duty cycle [95–97]. For instance, Gao et al. [95] reported that by increasing the electrical power from 0 to 160 W, the concentration of OH can be increased from 0 to 4 mmol/L,  $\text{H}_2\text{O}_2$  can be increased from 0 to 100 mg/L,  $\text{NO}_3^-$  can be increased from 1 to 225 mg/L, and,  $\text{NH}_4$  can be increased from 0 to 1.5 mg/L in the PAW. This increase can

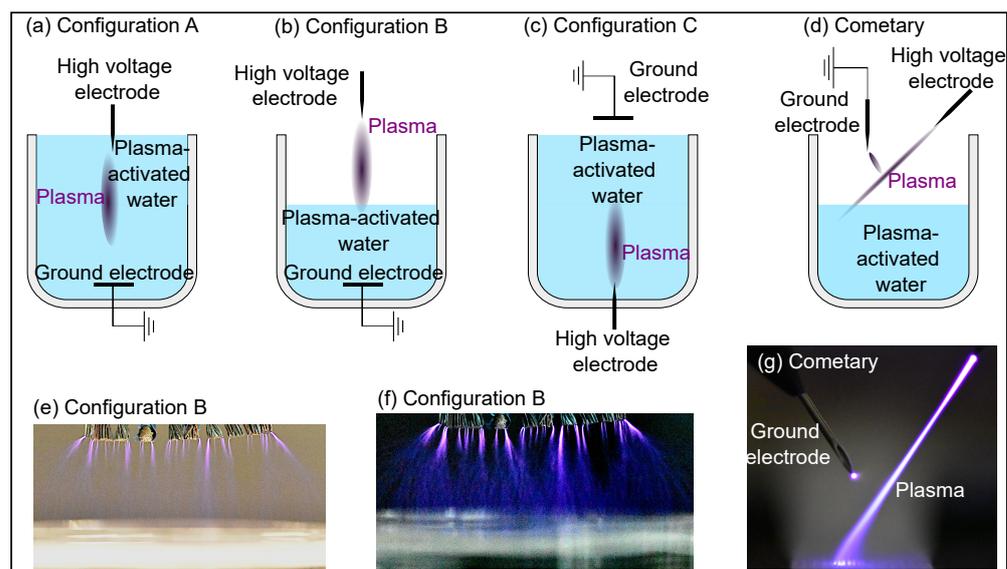
be attributed to an increase in energy density which enhances the rate of gas ionization [98]. An earlier study also demonstrated that changing the frequency and duty cycle of applied power can alter the efficiency of plasma-activated persulfate for sulfamethoxazole (SMZ) degradation in water [99]; an optimal duty cycle of 75% has shown the best SMZ degradation efficiency and the SMZ degradation efficiency increased from 77.3% to 89.2% when the discharge frequency was tuned from 150 Hz to 200 Hz. For the second operating parameter, longer treatment duration can lead to higher concentrations of reactive species. For instance, the concentration of  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in the PAW can be increased from 0 to 6.96 mg/L and 0 to 111.31 mg/L, respectively, after 60 min of treatment [100]. A separate study also showed that the concentration of  $\text{O}_3$  and  $\text{H}_2\text{O}_2$  in the PAW can be increased from 0 to 225 mg/L and 0 to 100 mg/L, respectively, after 12 min of treatment [101]. For the third operating parameter, a surrounding gas such as helium can alter the breakdown voltage, effective capacity, micro-discharge filaments, electron density, and surface charges, as compared to ambient air [102,103].

### 3.3. Corona Discharge

Compared to plasma jet and dielectric barrier discharge, corona discharge is simple and relatively easy to set up and operate [104]. The important setup parameters are (1) configurations of the electrodes and (2) geometry and material of the electrodes. The common configurations of the electrodes are: Configuration-A, Configuration-B, and Configuration-C, as illustrated in Figure 5a–c respectively. For Configuration-A (also known as liquid discharge), both electrodes are submerged in water and it can produce higher  $\text{H}_2\text{O}_2$  concentrations in the treated water. For Configuration-B (also known as gas discharge), the gas ionization takes place in the gaseous state and the generated reactive species are then dissolved into the liquid. Configuration-C (a hybrid gas–liquid discharge) can generate electrical discharge above and in the water concurrently; this enhances the production of reactive species through diffusion and direct interaction with water [105,106]. Corona discharge similar to plasma jet (known as cometary discharge) can be produced by arranging two needle electrodes, as shown in Figure 5d [107,108]. This plasma plume can be sustained without the feeding gas.

For the geometry of the electrodes, small radii of curvature are important for generation of high localized electric field strength. Generally, metal is preferable for the electrode material owing to its good electrical conductivity. However, a highly localized electric field focusing at the tip of metal electrodes and interaction of the metal with reactive species always causes erosion, which will deteriorate the operating lifetime [109,110]. An electrode material with a higher erosion rate (such as tungsten) can generate more ions in a liquid, increasing the electrical conductivity of PAW [109]. Additionally, due to the different degrees of sharpness and brittleness of the materials, tungsten and carbon electrodes required lower discharge currents as compared to aluminum, copper, and silver electrodes [111].

The important operating parameters are (1) electrical power and (2) treatment duration. For instance, the production of OH in the gaseous state is increased by increasing the electrical power [112]. The electrical power is directly proportional to the discharge power, i.e., higher discharge powers can produce PAW with higher electrical conductivities and concentrations of  $\text{H}_2\text{O}_2$  [113]. This can be attributed to the higher electric field and ionic wind velocity between the electrodes, leading to the increase in the formation of reactive species in the gaseous state as well as the transport of these reactive species to the liquid–air interface. As the density of these reactive species increases at the liquid–air interface, it accelerates the diffusion rate of reactive species into liquid [113–116]. Increasing treatment duration also increases the concentration of reactive species. For instance, the electrical conductivity of distilled water can be increased from 7.5 to 200  $\mu\text{S}/\text{cm}$  after 30 min of treatment, and  $\text{H}_2\text{O}_2$  concentration is increased from 0 to 60 mg/L after 14 min treatment [117].



**Figure 5.** Sketches illustrate the common configurations to generate corona discharge for the production of PAW. (a) Configuration-A, both electrodes are submerged in water; (b) Configuration-B, the high voltage electrode is above and the ground electrode is submerged in water; (c) Configuration-C, the ground electrode is above and the high voltage electrode is submerged in water; (d) Cometary, both electrodes are above water. Briefly, when a high voltage is applied across electrodes, the electric field strength near the electrode with a smaller radius of curvature (such as a sharp needle) increases significantly. The gas in surrounding the sharp needle is excited by the high electric field strength, breaking down into electrons and ions and forming plasma. Images (e,f) show the generation of atmospheric pressure plasma based on Configuration-B, where the high voltage electrodes consist of copper wire strands; image (g) shows the cometary discharge plasma.

## 4. Applications of Plasma-Activated Water

### 4.1. Disinfection and Decontamination

Due to the presence of various reactive species in PAW, the effects of these reactive species on microorganisms such as planktonic bacteria, biofilms, molds, fungi, and viruses have been conducted, as summarized in Table 1. For instance, in vitro study of PAW on planktonic bacteria such as *Aeromonas hydrophila*, *Enterobacter aerogenes*, *Escherichia coli*, *Hafnia alvei*, *Listeria innocua*, *Leuconostoc mesenteroides*, *Listeria monocytogenes*, *Pseudomonas deceptionensis*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Shewanella putrefaciens*, and *Salmonella Typhimurium*, have been demonstrated to be an effective technique for bacterial inactivation. Additionally, PAW is also effective at inactivating fungus spores, i.e., 75% and 85% reductions in *Aspergillus flavus* and *Aspergillus brasiliensis* spores can be observed after a 24 h and 30 min exposure to the PAW [118,119]. Viruses and bacteriophages such as the Escherichia virus T4,  $\phi$ X174 bacteriophage, Escherichia virus MS2, and Newcastle disease virus (NDV) can be also inactivated effectively after exposed to PAW. For instance, after being exposed to PAW, the morphology and structure of the DNA and RNA in the Newcastle disease virus were observed to be altered and damaged [120]. More recently, due to the COVID-19 outbreak, studies have been conducted on the use of PAW to inactivate the spike protein in the receptor-binding domain that is crucial during the infection process of the SARS-CoV-2 virus [121–123]. Their results showed that PAW can be used as an effective disinfectant to stop the infectious properties of SARS-CoV-2 through the inactivation of the spike protein present in its receptor-binding domain [122].

On bacterial inactivation, the effect of PAW on Gram positive bacteria was not as significant compared to Gram negative bacteria. This can be attributed to their cell structure, as the Gram positive bacteria have thicker cell walls [80]. Furthermore, the type of cell community can also affect the antimicrobial efficiency using PAW. For instance, biofilms are more resistant to PAW as compared to planktonic cells; this is because, on the biofilms, the

bacteria attach to each other and secrete an extracellular matrix that increases their resilience to stress [124]. Molds and yeast are generally more resilient towards PAW as compared to bacteria; this is because molds/yeast belong to the eukaryotes cell group that is equipped with a complete cell membrane for added protection to their nucleic acids [125]. The cell wall of fungus is also equipped with polysaccharides that form a complex capsule structure which contributes to a thicker cell wall and added protection to the cell [126].

The effectiveness of using PAW for decontamination of fresh produces such as lettuce, mung bean, shiitake mushroom, fresh-cut apples, fresh-cut pears, and meat such as chicken breast, beef mackerel cubes, and shrimps have also been studied extensively, as summarized in Table 2. Additionally, processed food such as bean curd and rice cake have been studied. As exposing foods to PAW cause minimal changes to these foods, its application in the food industry such as areas like decontamination, meat curing and the curtailment of pesticide residues has been investigated. In the case of microbial inactivation, the type of surface texture (of the foods) where microbes are attached has a significant effect on the effectiveness of the PAW. For instance, foods with rough surfaces can reduce the effectiveness of PAW, this is due to the inability of reactive species to penetrate those areas effectively [127]. A further study on using PAW to inactivate *E. aerogenes* attached on surfaces such as glass, grapes, limes, tomatoes, and spine gourd showed a similar finding in that the effectiveness of PAW is reduced when the surface roughness is increased [128]. The reactive species such as hydrogen peroxide, nitrite and nitrate of PAW used in the experiments listed in Tables 1 and 2 have concentration range around 0–900  $\mu\text{M}$ , 0–500 mM and 0–800 mM, respectively, while the volume of PAW used is in the  $10^{-3}$ – $10^0$  L range.

**Table 1.** Table summarizes the in vitro studies of the effectiveness of PAW for antimicrobial inactivation on contaminated surfaces. PAW was produced using different techniques, surrounding gas and treatment duration  $t_{\text{PAW}}$ ; as discussed above, the technique, surrounding gas,  $t_{\text{PAW}}$  and can affect the concentration of reactive species in PAW. The PAW was then applied on the microbe community at specific (antimicrobial inactivation) treatment duration  $t_e$ , to achieve the desired reduction. DBD is referred to dielectric barrier discharge.

Technique	$t_{\text{PAW}}$	Microbe Community	$t_e$	Microbe	Reduction
Corona; Air	60 min	Biofilm	30 min	<i>S. aureus</i> [129]	4.74 log CFU/mL
DBD; Air	20 min	Fungus spores	24 h	<i>A. flavus</i> [118]	0.6 log CFU/mL
DBD; NO	10 s	Planktonic	5 min	<i>E. coli</i> O157:H7 [130]	3.10 log CFU/mL
	5 min	Planktonic	10 s	<i>L. monocytogenes</i> [130]	4.13 log CFU/mL
DBD; Ar with 1% Air	2 min	Bacteriophages	1 h	<i>E. virus</i> MS2 [131]	~11 log PFU/mL
				<i>E. virus</i> T4 [131]	>11 log PFU/mL
				$\phi\text{X174}$ bacteriophage [131]	>10 log PFU/mL
DBD; He with 1% O <sub>2</sub>	30 min	Planktonic	30 min	<i>L. monocytogenes</i> [124]	5.3 log CFU/mL
		Biofilm		<i>L. monocytogenes</i> [124]	3.2 log CFU/mL
		Planktonic		<i>S. Typhimurium</i> [124]	5.8 log CFU/mL
		Biofilm		<i>S. Typhimurium</i> [124]	3.9 log CFU/mL
Gliding arc; Humid air	5 min	On stainless steel	30 min	<i>H. alvei</i> [132]	5.36 log CFU/mL
				<i>L. mesenteroides</i> [132]	4.69 log CFU/mL
				<i>S. cerevisiae</i> [132]	3.07 log CFU/mL
Plasma jet; Air	60 s	Planktonic	6 min	<i>E. coli</i> O157:H7 [133]	3.7 log CFU/mL
	60 s	Planktonic	6 min	<i>S. aureus</i> [133]	2.3 log CFU/mL
	90 s	Planktonic	10 min	<i>P. deceptionensis</i> CM2 [134]	5 log CFU/mL
	3 min	Fungus spores	30 min	<i>A. brasiliensis</i> [119]	0.82 log CFU/mL
	5 min	Biofilm	5 min	<i>P. fluorescens</i> [135]	6 log CFU/mL
	5 min	Planktonic	10 min	<i>E. aerogenes</i> [128]	1.92 log CFU/mL
	5 min	Planktonic	0.5–24 h	<i>A. hydrophila</i> [80]	>7.12 log CFU/mL
	5 min	Planktonic	0.5–24 h	<i>E. coli</i> [80]	>6.79 log CFU/mL
	5 min	Planktonic	0.5–24 h	<i>L. innocua</i> [80]	>6.15 log CFU/mL
	5 min	Planktonic	0.5–24 h	<i>P. fluorescens</i> [80]	>6.86 log CFU/mL
	5 min	Planktonic	0.5–24 h	<i>S. aureus</i> [80]	>4.52 log CFU/mL
5 min	Planktonic	0.5–24 h	<i>S. putrefaciens</i> [80]	>7.06 log CFU/mL	

**Table 2.** Table summarizes the effectiveness of PAW for antimicrobial inactivation on contaminated foods. PAW was produced using different techniques, surrounding gas and treatment duration  $t_{PAW}$ ; as discussed above, the technique, surrounding gas,  $t_{PAW}$  and can affect the concentration of reactive species in PAW. The PAW was then applied on foods at specific (antimicrobial inactivation) treatment duration  $t_e$ , to achieve the desired reduction. DBD is referred to dielectric barrier discharge.

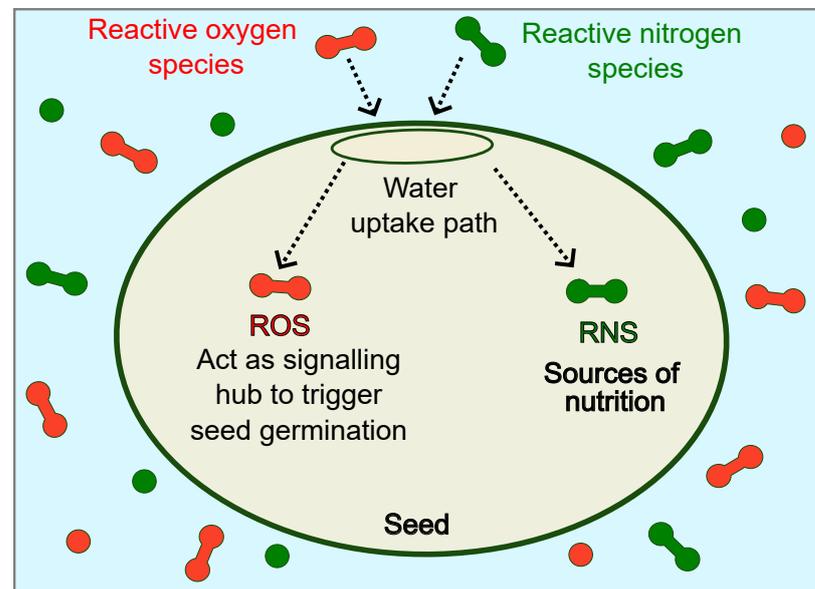
Technique	$t_{PAW}$	Food	$t_e$	Microbe	Reduction
DBD; Air	10 min	Fresh shrimp	9 days	Natural microflora [136]	1.32 log CFU/g
	10 min	Lettuce	5 min	<i>L. innocua</i> [137]	2.4 log CFU/g
	10 min	Lettuce	5 min	<i>P. fluorescens</i> [137]	>6 log CFU/g
	20 min	Chicken breast	20 min	<i>S. aureus</i> [138]	2.09 log CFU/g (MRSA)
	20 min	Chicken breast	20 min	<i>S. aureus</i> [138]	2.29 log CFU/g (MSSA)
	20 min	Rice cake	20 min	<i>E. coli</i> O157:H7 [139]	2.01 log CFU/g
	20 min	Rice cake	20 min	<i>L. monocytogenes</i> [139]	2.02 log CFU/g
	20 min	Rice cake	20 min	<i>S. typhimurium</i> [139]	2.08 log CFU/g
	20 min	Rice cake	20 min	<i>C. albicans</i> [139]	1 log CFU/g
	20 min	Rice cake	20 min	<i>P. chrysogenum</i> [139]	1.97 log CFU/g
Plasma jet; Air	30 s	Sprouts	30 mins	Natural microflora [140]	2.32 log CFU/g (bacteria)
	30 s	Sprouts	30 mins	Natural microflora [140]	2.84 log CFU/g (yeast/moulds)
	60 s	Beef	until frozen	Natural microflora [141]	1.98 log CFU/g (fungus/yeast)
	90 s	Bean Curd	30 min	Natural microflora [142]	1.26 log (bacteria)
	90 s	Bean Curd	30 min	Natural microflora [142]	0.91 log (yeast/molds)
	10 min	Apple	5 min	Natural microflora [143]	1.05 log CFU/g (bacteria)
	10 min	Apple	5 min	Natural microflora [143]	0.64 log CFU/g (molds)
	10 min	Apple	5 min	Natural microflora [143]	1.04 log CFU/g (yeasts)
	10 min	Bean curd	24 h	<i>E. coli</i> O157:H7 [144]	>2 log CFU/g
	10 min	Bean curd	24 h	<i>L. monocytogenes</i> [144]	~0.5 log CFU/g
	10 min	Bean curd	24 h	<i>S. enteritidis</i> [144]	>2 log CFU/g
	10 min	Bean curd	24 h	<i>S. typhimurium</i> [144]	>2 log CFU/g
	15 min	Fresh mackerel	30 min	<i>P. fluorescens</i> [145]	0.4 log CFU/g
	20 min	Mushroom	20 min	Natural microflora [146]	>1 log CFU/g
	20 min	Shell eggs	1 min	<i>S. enteritidis</i> [147]	>4 log CFU/egg
	30 min	Kiwifruit	8 days	<i>S. aureus</i> [148]	1.8 log CFU/g
60 min	Chicken breast	12 min	<i>P. deceptionensis</i> CM2 [149]	1.05 log CFU/g	

#### 4.2. Enhancement in Seed Germination

Enhancing seed germination is critical for increasing agricultural productivity, as it is a key factor in determining crop yield frequency and growth rate, which are essential for ensuring food availability [150,151]. The seed germination process involves three stages: water absorption to initiate metabolism, biological metabolism process, and termination upon radicle protrusion [152–154]. Water absorption and seed biological metabolism processes are important for increasing seed germination rate. Increasing the water absorption rate occurs mainly via physical mechanisms. Common techniques are: (1) increase the temperature [155–157], (2) increase the hydrostatic pressure [158,159], and (3) exposure to ultrasound [4,160–163]. On the other hand, increasing seed metabolism occurs mainly via chemical and biological mechanisms. Common techniques are chemical [164–168] and exogenous phytohormone [169–172] treatments for embryo development. Nonetheless, these techniques can create risks of byproduct contamination during the seed germination process and harm the environment.

PAW contains a high concentration of reactive species, which can enhance biological reactions. The effectiveness of PAW to increase germination is depended on the concentrations of ROS and RNS, as illustrated in Figure 6. The ROS act as signaling hubs, triggering transduction events in cellular responses related to seed germination, whereas the RNS are fundamental in plant nutrition, regulating cellular redox status and the synthesis of amino acid, proteins and chlorophyll [13,16,173,174]. For instance, PAW produced by plasma jet with argon–oxygen feeding gas can increase wheat seed germination process, achieving a 100% germination rate for treated seeds as compared to 97% for seeds without treatment [175]; PAW produced by plasma jet with air feeding gas can increase the pea seed germination by 37% [176]; PAW produced by dielectric barrier discharge with argon–nitrogen–oxygen surrounding gas can increase the *Lactuca sativa* L. seeds germination potential by 117% [177]. Nonetheless, not all reactive species in high concentrations are

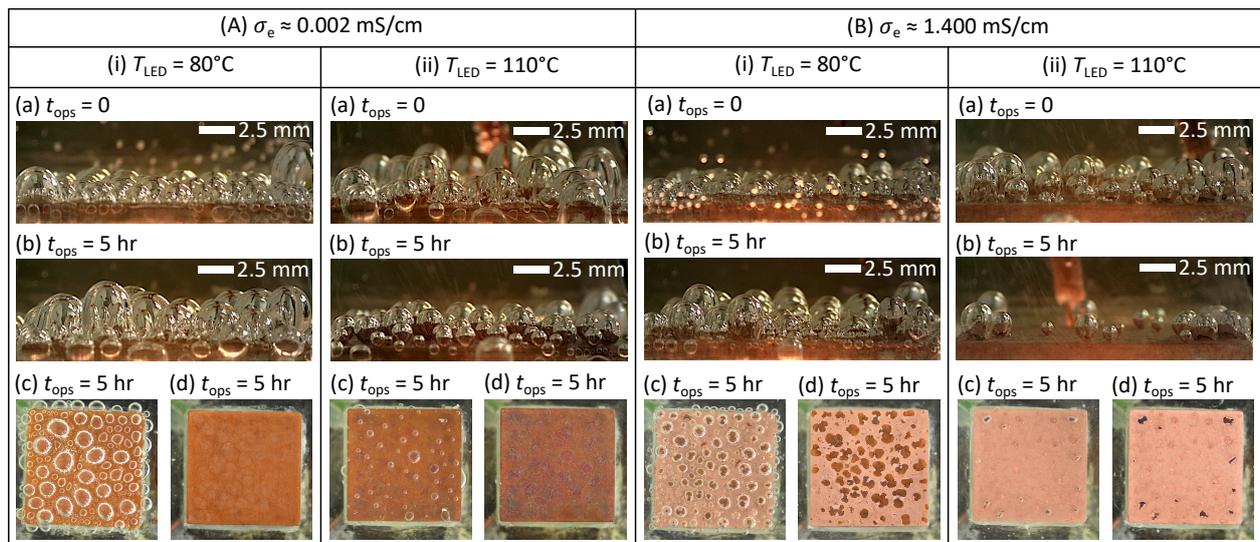
favorable for enhancing seed germination. For instance, excessive concentration of ROS can lead to high abiotic stress and thus affect seed viability [4,14,15].



**Figure 6.** The sketch illustrates the concept of the use of PAW to enhance seed germination. ROS can act as a signaling hub to trigger the biological metabolism process during germination and RNS can act as a primary source of nutrition.

#### 4.3. Enhancement in Surface Cooling and Reduction in Surface Oxidation

Due to the presence of reactive species in PAW, the physical properties of the solutions can be altered significantly by increasing the concentrations of these reactive species, i.e., by prolonging the treatment duration such as to increase the electrical conductivity of the solutions. As can be seen in Figure 1, by increasing the electrical conductivity of PAW, the density and viscosity are increased, whereas the surface tension is reduced [34]. A similar trend was reported by Ekanayake et al. [178,179], in which they observed higher evaporation rates for seawater treated with atmospheric pressure plasma. By exploiting this interesting change in the surface tension of PAW for application in surface cooling in the nucleate boiling regime (phase-change heat transfer), Low et al. reported an increase in droplet evaporation rate (using PAW with electrical conductivity  $\sigma_e \approx 1.4$  mS/cm) by up to 40% as compared to deionized water ( $\sigma_e \approx 0.002$  mS/cm). Additionally, in the nucleate and transition boiling regimes (approximately 130–150 °C), up to 220% enhancement of the reduction in surface temperature, can be obtained by using PAW as compared to deionized water [5]. Similar enhancement in surface cooling can also be obtained for the pool cooling system, i.e., up to 66% increase in pool evaporation rate by using PAW as compared to deionized water can be achieved [6]. Peculiarly, when applying the concept on a steady-state, phase-change heat transfer cooling system that operates in the nucleation boiling regime, with the PAW ( $\sigma_e \approx 1.4$  mS/cm), they observed that not only the heat transfer coefficient can be increased by up to 73%, but the surface oxidation (after a 5 h operation) of the heat source (a copper material) is also reduced (see Figure 7) in comparison to deionized water ( $\sigma_e \approx 0.002$  mS/cm) [6]. Further studies on the effects of PAW aging on the cooling performance and the role of reactive species in altering its physical properties are needed; an earlier study showed that the stability of PAW can be maintained for up to 2 weeks by considering high reduction potential metals during the treatment [180].

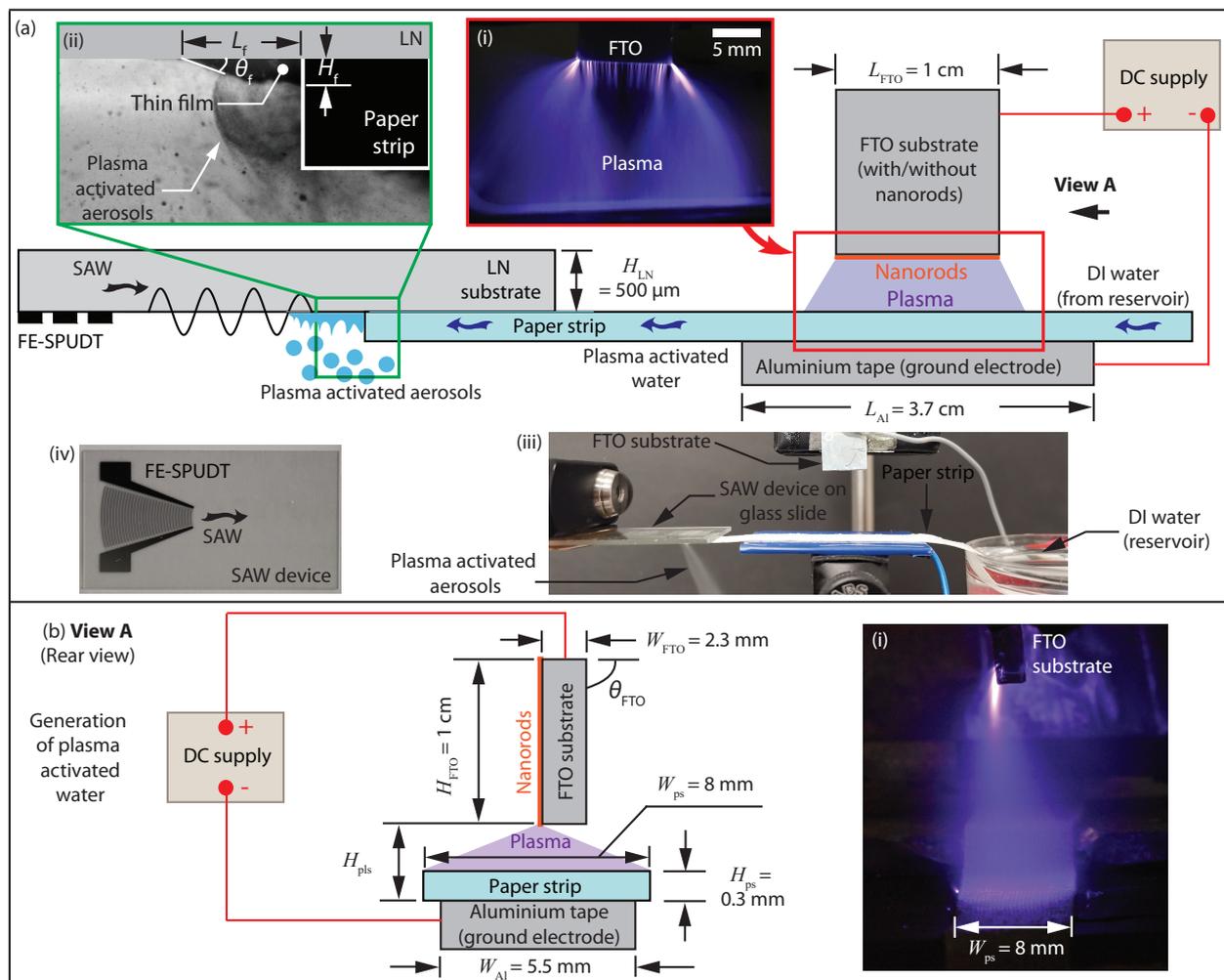


**Figure 7.** By using PAW ( $\sigma_e \approx 1.4$  mS/cm) as coolant atop the heat source (a copper material) at the steady-state surface temperature of  $110^\circ\text{C}$ , after 5 h of continuous operation, only a small fraction of copper oxides were formed on the heat source surface (B-ii-d), as compared to using deionized water ( $\sigma_e \approx 0.00$  mS/cm), where the entire surface of the heat source was oxidized (A-ii-d). From [6]. Copyright 2023 Author(s), licensed under a Creative Commons Attribution (CC BY-NC-ND 4.0) License.

### 5. Miniaturization for In Situ Generation of Plasma-Activated Aerosols

Although studies have demonstrated the effectiveness of PAW for application in surface disinfection, commercial products based on PAW are limited. This can be attributed to the challenge of making the plasma system portable by miniaturizing the plasma generator such that the PAW can be applied directly on the contaminated surfaces without the need for storage. As highlighted above, proper storage of PAW at lower temperatures is important to ensure no significant degradation of the reactive species in PAW. For instance, the effectiveness of microbial inactivation can be reduced by up to 20% and 80% when the PAW is stored at  $-80^\circ\text{C}$  and  $25^\circ\text{C}$ , respectively, for 30 days [24]. As such, there is a need to develop an integrated miniaturized plasma generator and portable nebulizer for the in situ generation of plasma-activated aerosols that can be applied directly on contaminated surfaces.

The first such system was reported by Wong et al. [181]. In their study, the plasma generator was based on the cometary discharge plasma (Figure 5d), whereas the nebulizer was based on surface acoustic wave device. By spraying the plasma-activated aerosols directly on the contaminated surfaces with *E. coli*, they obtained a percentage reduction of up to 96%. To further miniaturize the plasma generator, a nanoscale plasma generator was developed [182]. Briefly, zinc oxide nanorod carpets coated with aluminum that mimic a conglomeration of nanoneedles were used to generate plasma (see Figure 8), leading to a considerable increase in the reactive species in the plasma-activated aerosols. Although the in situ production of plasma-activated aerosols via the nanoscale plasma generator is a promising technique, it is challenging to deposit plasma-activated aerosols onto a specific contamination area. To address this limitation, DC electro spray can be used to generate plasma-activated aerosols; due to the strong electric field, these finer plasma-activated aerosols can be deposited on the contaminated surface [34].



**Figure 8.** (a) Sketch illustrates the concept of an integrated nanoscale plasma generator and a portable acoustic nebulizer. Briefly, zinc oxide nanorods coated with a thin layer of aluminum were used to generate atmospheric pressure plasma (a-i) to treat the water transported within the paper strip (a-iii). When the PAW reaches the surface of the lithium niobate (LN) substrate (a-iv), due to the high surface acceleration, the liquid film destabilizes and produces plasma-activated aerosols (a-ii). (b) The rear view of the nanoscale plasma generator and (b-i) the plasma above the paper strip. From [182]. Copyright 2022 Author(s), licensed under a Creative Commons Attribution (CC BY 4.0) License.

## 6. Conclusions

PAW is an up-and-coming technology that can be used in applications such as antimicrobial activity, enhancement of seed germination, and improvement of surface cooling in the nucleate boiling regime. This is mainly attributed to the unique physicochemical properties and biochemical activities that it possesses after being treated with atmospheric pressure plasma. Here, in the first part of this review, we discussed the physicochemical properties of PAW and highlighted the common RONS in PAW that are responsible for the above three applications. Given that different techniques to generate the atmospheric pressure plasma have significant impact on the concentrations of the RONS in PAW, in the second part, we discussed the three commonly used techniques (plasma jet, dielectric barrier discharge, and corona discharge) to generate atmospheric pressure plasma. Finally, in the third part, we discussed the recent development in the use of PAW for the above three applications, such as the recently demonstrated concept of an integrated nanoscale plasma generator and acoustic nebulizer; this integrated portable system not only enables the in situ production of PAW, but also the generation of plasma-activated aerosols that can deposit directly on contaminated surfaces. While PAW has demonstrated effectiveness in

these applications, a thorough safety assessment of long-term usage must be conducted before its application in the food or biomedical industries.

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