GRADUATE REVIEW ARTICLE

Influence of Feeding Gases on the Composition of Plasma Activated Water

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ABSTRACT

As we are all aware that "PLASMA" is the fourth state of matter and about 99% of the universe comprises of plasma. Plasma invariably consists of essential reactive oxygen and nitrogen species which are necessary for agricultural purposes thus making it an interesting subject for research. When water is exposed to plasma arc, its composition changes and forms Plasma Activated Water (PAW). Research studies have proved PAW to be an effective disinfectant and also providing imperative nutrients to plants. This paper reviews the impact of feeding gases such as Air, Ammonia, Argon, Nitrogen, Helium, Oxygen and Carbon dioxide on PAW composition. Hydrogen peroxide, nitrates, nitrites and pH value are the four key aspects of PAW which decide its influence. H_2O_2 helps in bacterial inactivation whereas nitrates and nitrites are a source of nutrients. It is known that nitrites decompose rapidly in water and form compounds that promote bacterial inhibition. Here the impact of using Air, Ammonia, Argon, Nitrogen, Helium, Oxygen and Carbon dioxide is being reviewed and studied. More specifically, the concentration of major Reactive Oxygen and Nitrogen Species (RONS) formed in the process and the physical properties of PAW at various atmospheres are is discussed in detail.

Keywords: Plasma activated water (PAW), Hydrogen peroxide, Feeding gases, Reactive Oxygen and Nitrogen Species (RONS)

1   Introduction

Chemical pesticides and fertilizers enter the food chain and harm the creatures/humans that consume these artificially cultivated food grains. Moreover, these chemicals remain in water and soil for many years leading to environmental pollution. As the world population is increasing manifold every second, it is necessary to create innovative approaches for food production in order to meet the global demand. Hence, PAW can be used as an alternative for conventional nitrogen fertilizers.

Basically, plasma is the fourth state of matter and composed of positive and negative ions, electrons, excited and neutral atoms, free radicals, molecules in the ground and excited states and UV photons [1]. Plasma Activated Water (PAW) is obtained on dissolving plasma into water in two ways i.e., with the help of electricity. First method involves inducing plasma directly into water and the second method is through direct contact of plasma streaming with the water. The second method is the most convenient one used for mass production of PAW by atmospheric pressure plasma. According to Yun Sik Jin *et al* (March 2020) the direct method when used showed good results as compared to indirect method. The measurement results of pH, conductivity and nitrate concentration of the solution showed that the direct type had superior characteristics in terms of PAW production compared to the indirect type. This big difference is because the direct type provides a structure in which the plasma and water interact closely [2]. According to Jan Čech *et al* the plasma activated water produced by the hydrodynamic cavitation plasma jet (HCPJ) for industrial-scale generation of biologically active environments at high flow rates of several m3/h proved to be the best with long term biological effects in inhibiting algae and cyanobacteria. It was noted that PAW produced by HCPJ (Figure 1) could destroy the metabolic activity of cyanobacterial cells and kill the cyanobacterial population in 24 h after the treatment [3].
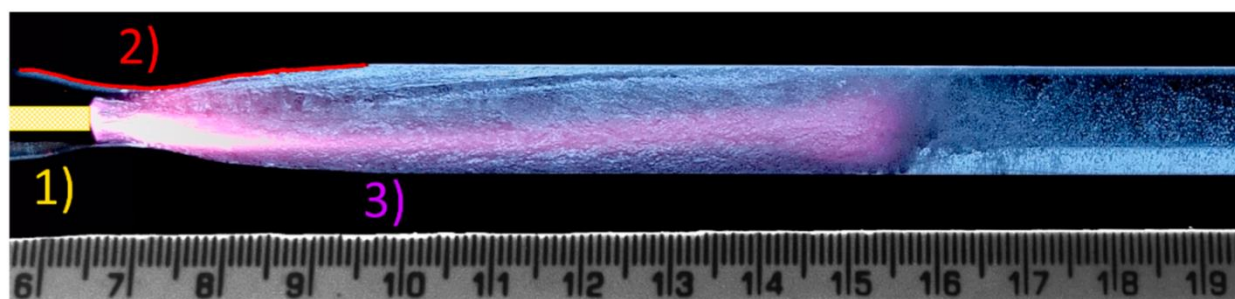
**Figure 1:** *The setup is called a hydrodynamic cavitation plasma jet (HCPJ)*

A HCPJ generated in a Venturi nozzle with the flow of 0.55 m3/h at sub-atmospheric backpressure of 40 kPa. Plasma was initiated using an HV sine-wave generator with the frequency of 65 kHz. HV measured at electrodes was 6.4 kV peak-to-peak. Power was set to 400 W measured at the HV power supply input. Exposure time was 1/20 s. The positions of the HV electrode (1) and outer contour of the Venturi nozzle (2) are indicated by the yellow and red colors, respectively. The discharge channel (3) was generated inside a cavitation cloud. The electrodes were separated by approximately 16 cm. (Courtesy: [3])

N. Punith *et al* stated that Ozone and hydrogen peroxide are major reactive oxygen species whereas nitrites, nitrates and peroxynitrites are major reactive nitrogen species. Nitrates produced in plants can serve as an alternative source of nitrogen for enhancing the growth of plants [4]. According to J. Julak *et al* Nitrates play two main roles - as a nutrient, it is assimilated by plant enzymes leading to the production of amino acids and nitrogen compounds; and as a signal molecule it can control numerous aspects of plant development and metabolism. The acidification of PAW depends on the nature of atmosphere surrounding the discharge [5].

According to John E. Foster the hydrogen peroxide is formed when two OH radicals combine and it should be noted that a free radical, such as OH, is essentially a molecule with one or more unpaired electrons, which makes it particularly reactive. OH has an oxidation potential second only to fluorine, which is toxic, and twice that of chlorine. It should be pointed out that the oxidant chlorine tends to be selective in what it actually oxidizes whereas OH is indiscriminant [6].

Pradeep Lamichane and others stated that plasma is used in various fields to generate various types of reactive oxygen species (ROS) like hydroxyl radical (OH), atomic oxygen (O), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2), reactive nitrogen species (RNS) like excited (N_2^+), and amine (NH_2) and reactive oxygen and nitrogen species (RONS) like nitric oxide (NO), nitrite (NO_2), nitrate (NO_3), and peroxynitrite (ONOO⁻) [7]. These species are produced through the interaction of energetic electrons, ions and excited species with ambient gases. Thus, the feed gas plays a significant role in determining the species in water.

Various research studies have been made regarding the usage of air, oxygen, helium, carbon dioxide, argon, ammonia, hydrogen and nitrogen as feed gas during preparation of plasma activated water (PAW). This review article aims to present the impact of these feed gases on the composition of plasma activated water. Moreover, Evanthia Tsoukou *et al* in their study on the impact of non-buffered Plasma Activated Liquids (PAL) such as Plasma Activated Saline (PAS) and Plasma Activated Water (PAW) showed their anti-bactericidal efficacy and stability of PAW for up to 18 months when stored at the lowest temperatures (Figure 2) [8].
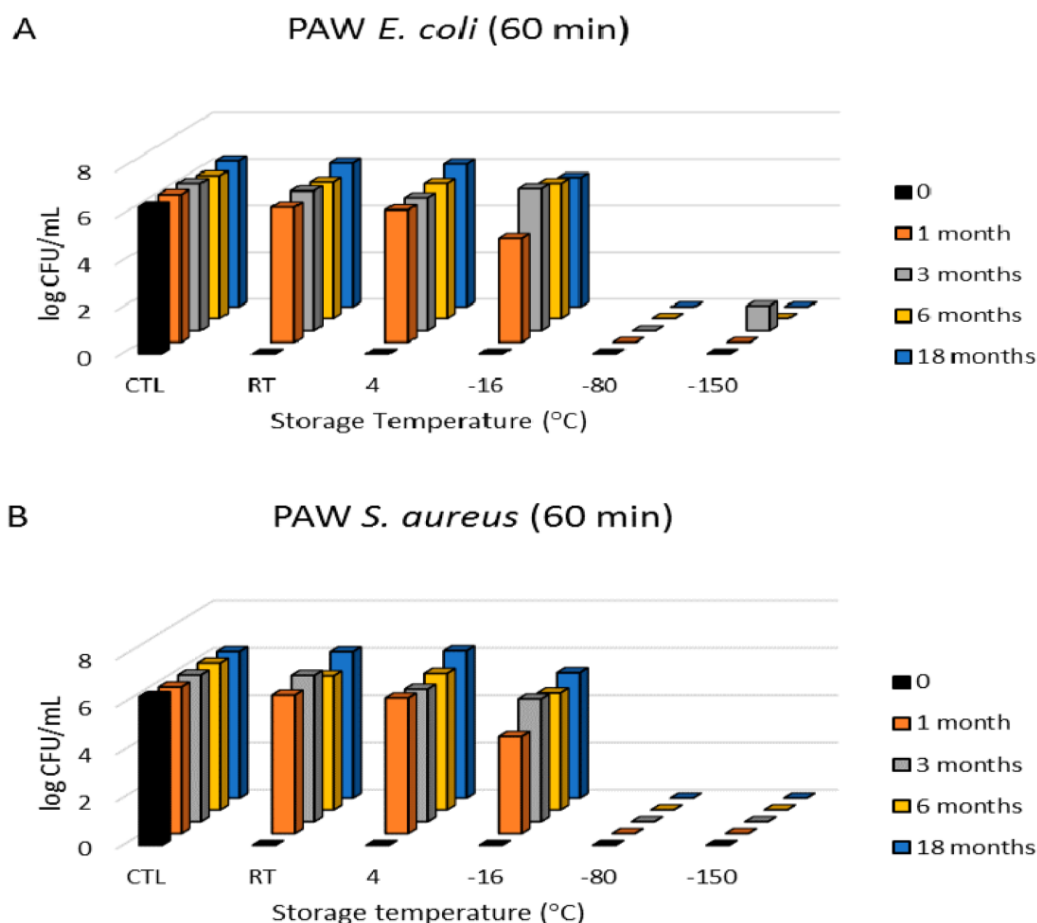
Figure 2: *Microbial inactivation of E. coli and S. aureus at 60 min contact time, treated with PAW stored at different temperatures for up to 18 months. Graphs represent averages of two independent plasma treatments (Sets 1 and 2); standard deviations can be found in Supplementary Table S1. (A) PAW E. coli (60 min), (B) PAW S. aureus (60 min) (Courtesy [8]).*

2 Feeding Gases

2.1 Air

Rohit Thirumdas *et al* observed that air used as a working gas adopting various methods such as Plasma microjet, dielectric barrier discharge (DBD), Gliding arc and DBD with hallow electrodes produced PAW with pH ranging between 1.9 to 2.8. It was also stated that formation of Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) during formation of PAW contributed to increase in conductivity of PAW. This PAW would help in microbial disinfection, decontamination, enhancing seed germination and plant growth of seeds and disinfection. ROS acted as a signal in seed dormancy alleviation [9].

Renwu Zhoua *et al* stated that the microbial inactivation of air-PAW is effective because it contains H_2O_2 and NO_2^- in prerequisite concentrations that can generate compounds like peroxynitrous acid and peroxynitric acid which exhibit antibacterial effect under acidic conditions. They performed the activation

by using different feeding gases like N_2, He, O_2 and air on sterile distilled water and reported that air PAW had lower pH compared to other PAW [10].

According to the findings of N. Punith *et al* the wet weight is a sign of overall health of a plant and plants with influential wet weight are healthier. It was perceived that wet weight of plants increased when they were treated with air-plasma and this causes primarily on the account of formation of the reactive nitrogen species [2]. Air-PAW enhanced the germination of mung bean seeds (Figure 3). and seedling growth and the superoxide dismutase activities were found to be higher, when this PAW was given to mung bean seedlings. It furthermore helped to decrease the malondialdehyde, a colourless liquid in plants that is a maker for oxidative stress [10]. Thus, Air-PAW has the potential to increase the yield of plants and has the highest oxidation reduction potential, which is an imperative aspect for the microbial inactivation.
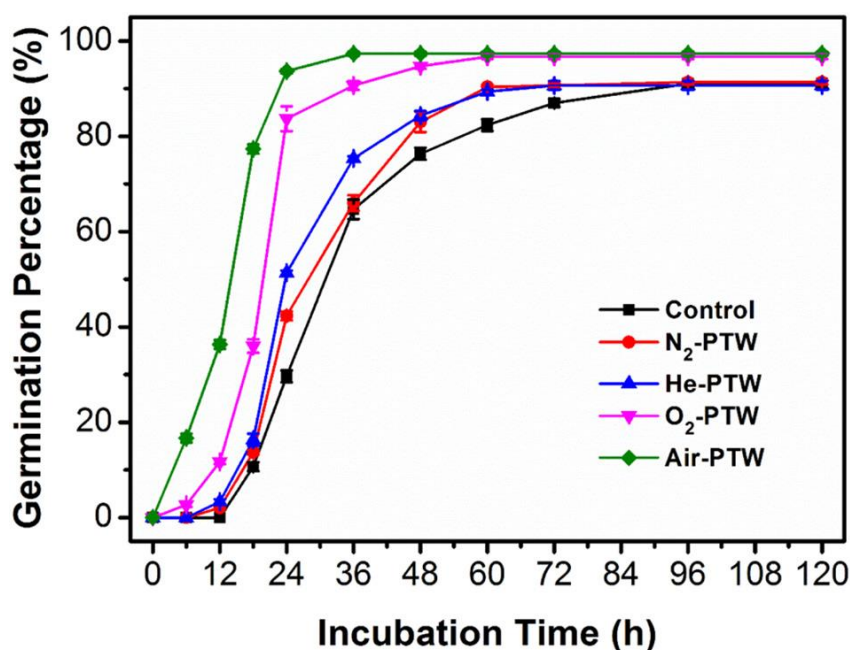
Figure 3: *The germination percentage of mung bean seeds treated with He-PTW, N2-PTW, O2-PTW and Air-PTW as a function of incubation time (Courtesy [10].*

2.2   Ammonia

Hydrogen peroxide is a vital compound which controls the chemical and physiological behaviours in cells, such as signalling pathways. However, its high concentration results in oxidative stress. So, it is necessary to control the H_2O_2 content in PAW.

Pradeep Lamichhane *et al* noted that the argon mixed with ammonia (NH_3) feed gas is capable of monitoring the concentrations of H_2O_2 in PAW by applying the nitrification method. Nitrification takes place in two steps, the first stage involves the oxidation of NH_3 to NO_2^-, and in second step the NO_2^- formed is oxidized to NO_3^-. This can lead to more production of NO_2^-, NO_3^-. Plants absorb them to make proteins and it enhances the process of photosynthesis. Experiments were also done to study the effect of argon with NH_3 and it was observed that only argon impacts on production of reactive species. When Ar/H_2O was used as a working gas, the concentrations of H_2O_2 and NO_3^- were 14ppm and 1ppm, the H_2O_2 content declined to 4ppm and NO_3^- content increased to 6ppm after the addition of NH_3 was done into the feed gas. The pH value of PAW rose when NH_3 was added. The flow rate of NH_3 was pretty high than the electron density, so most of the ammonia didn't decompose and remain unchanged in water (i.e. they remained alkaline in nature). Hence the pH value was found to be higher when Ar/NH_3 was used but was lower in case of Ar/H_2O [7].

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2.3 Argon

According to Kristína Trebulová *et al* using conventional gases such as air or argon it is possible to produce Reactive oxygen species (ROS) and Reactive nitrogen species (RNS), ozone, oxides and peroxides in a very short time. In addition, non-thermal plasmas efficiently produce ultraviolet (UV) radiation in the UVC region and vacuum ultraviolet (VUV) radiation, which have strong antimicrobial effects. Devices effectively producing such type of plasma are based on the corona discharge, dielectric barrier discharge (DBD), atmospheric pressure glow discharge, plasma beam or nanosecond pulsed discharge.
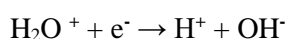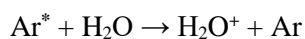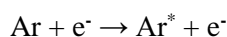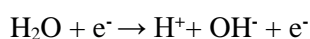
It was noted that usage of pure argon as career gas lead to sweeping in of molecules contained in the surrounding air (oxygen, nitrogen, water vapor, etc.) into the plasma flow wherein they were excited or ionized resulting in the presence of RONS such as nitrogen monoxide radical (NO), hydroxyl radical (OH), atomic oxygen (O), peroxynitrite molecule (ONOOH) or ozone (O3) in the plasma torch [11].

J Julák *et al* prepared PAW in the atmosphere of Ar, air, CO_2 and N_2. The H_2O_2 content obtained in Ar-PAW was 2-3 times higher than other PAWs. It persisted the activity until 1 year, up to 1/80 dilution and it was attributed to higher concentrations of H_2O_2. It was further stated that H_2O_2 compound was biologically active agent among the other feasible active species in PAW which implies that it had higher concentrations of OH radicals and peroxonitrous acid also exhibits its impact on bacterial calls before it gets decomposed [5].

Ruonan Ma *et al* used the PAW obtained on strawberries to verify its action on staphylococcus aureus (which is a gram-positive bacterium that causes food poisoning) inoculated on strawberries and concluded that PAW had inhibited the growth of majority of bacteria when it was exposed for 15 minutes on strawberries. The Ar-PAW didn't disturb the colour, firmness and pH of the strawberries and moreover it also restrained the fungal growth on the fruit. They asserted that plasma treatment time and activation time influenced the regulation of bacteria. A remarkable rise in the value of the pH was detected after storing it for 4 days. This rise in pH affects the amount of nutrients in plants and contributes to the growth of plant [12].

Konstantin Artem'ev *et al* made PAW with piezoelectric discharge and the acquired Ar-PAW preserved its effect for at least 8 days. As the exposure time had an upsurge, the conductivity and ORP values also increased. The pH value of Ar/H_2O plasma treated water dropped after energy consumption, implying that water had undergone acidification. It showed that the distilled water is mainly produced with RONS (mainly H_2O_2 and NO_2^-), which can be stored (the concentration changes only slightly) for at least 8 days [13].

The reactions can be summarized as mentioned by Pradeep Lamichhane *et al* [7] as follows:

$$H_2O + e^- \rightarrow H^+ + OH^- + e^-$$

$$Ar + e^- \rightarrow Ar^* + e^-$$

$$Ar^* + H_2O \rightarrow H_2O^+ + Ar$$

$$H_2O^+ + e^- \rightarrow H^+ + OH^-$$

$$OH^- + OH^- \rightarrow H_2O_2$$

A Bacteriophage is a type of virus that infects bacteria. A "Bacteriophage" literally means "bacteria eater", since bacteriophages destroy their host cells.

Based on their studies Li Guo *et al* proposed a model of bacteriophage inactivation by plasma and plasma-activated water (Figure 4). They revealed that plasma-generated reactive species, especially singlet oxygen, efficiently inactivated different kinds of bacteriophages such as double stranded DNA, Single stranded DNA and RNA bacteriophages in water by damaging the nucleic acids and proteins [14]
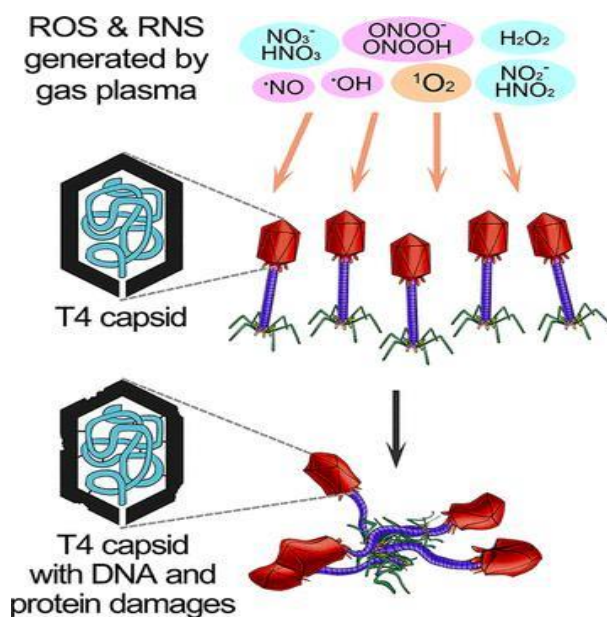
Figure 4: *The inactivation of bacteriophage T4 by ROS and RNS of plasma (Courtesy: Li Guo et al [14]).*

2.4   Nitrogen

According to Julak *et al* the acidity of PAW is caused by the presence of reactive nitrogen species. In nitrogen atmosphere, the PAW becomes more acidic due to the formation of high content HNO_3 and it persists its activity even when it is diluted whereas H_2O_2 concentration gradually decreases [5].

Priyanka Shaw *et al* obtained PAW with distilled water, DBD and tested the effect of N_2, N_2+H_2O, N_2+HNO_3 feed gases on PAW composition. The H_2O_2 concentration in (N_2+H_2O) PAW was found to be more since it had high OH intensity. In (N_2+HNO_3) PAW, higher amount of NO was noticed than in other circumstances, as a consequence it had greater concentrations of NO_2^- and NO_3^- and it inactivated E. Coli bacteria to the maximum extent. Therefore, the H_2O vapour system resulted in higher percentage of Reactive Oxygen Species (ROS) but the HNO_3 vapour system yielded Reactive Nitrogen Species (RNS) as well as ROS species [15].

According to Chae Bok Lee *et al* the impact of N_2-PAW on inhibition of E. Coli is not as much of (N_2+H_2O)-PAW and (N_2+HNO_3)-PAW. The effect of RNS on biological systems depends on its concentration and these RNS react with biomolecules in human body to induce cascade process [16].

Renwu Zhoua *et al* stated that the N_2 plasma jet showed the weakest OH emission and the ORP values of N_2-PAW were relatively lower than O_2 and air PAWs. Electrical conductivity increased dramatically after 10 minutes of activation because various ROS and other reactive chemical species were derived from chemical reactions between water molecules and plasma electrons [10]. Joanna Pawlat *et al* produced PAW by using gliding arc discharge in nitrogen atmosphere with synthetic tap water and phosphate buffered solution (PB) as liquid samples. They observed low pH value and high conductivity for tap water and it was concluded that the conductivity of water increased with plasma treatment. The NO_2^- and NO_3^- species were higher in PB solution while tap water had more H_2O_2 content and hence it showed bactericidal effect [17]. While Priyanka Shaw *et al* [15] asserted that bacterial inactivation effect was due to O_2^- which was generated by O_2NOOH, Joanna Pawlat *et al* [17] claimed that peroxynitrates relieved PAW from bacteria. ONOOH produce OH and NO radicals which contributed to bactericidal effect. N_2^- plasma activated solutions with 5 minutes of activation reduced the population of gram positive S. Epidermis bacteria on stainless steel, silicone and Polyethylene Terephthalate (PET) surfaces. This N_2-PAW can be utilized for surface decontamination. The inhibitory effects were instigated by the failure of anti-oxidant defence machinery under high oxidative stress caused by the reactive oxygen species [16]. It may be noted that

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nitrogen medium is eminently supportable for the formation of reactive nitrogen species as determined by many researchers.

2.5 Helium

Argon and Helium almost show similar impact on composition of PAW. Zhang *et al* used tap water, fertilizer and demineralized water as liquid samples for plasma activation with DBD and compared their effect on lentil seeds. Results showed that tap water and demineralized water attained 160μM of hydrogen peroxide while in liquid fertilizer it was estimated to be 350μM. Tap water and demineralized water displayed only 450μM and 50μM of nitrate concentration. In contrast, nitrate concentration in liquid fertilizer got promoted to 10000μM after plasma activation [18]. Nitrates lead to the production of NO, which is involved in the seed dormancy. In another study conducted by Renwu Zhou *et al* for generation of He-PAW, low concentrations of O_3 and NO_2 were observed. NO concentration in PAW was limited to only 12ppm due to its conversion into NO_2 when it reacted with O_2. The collision between electrons and water vapour gave rise to the formation of OH species in He-PAW and as a result, it exhibited higher pH value than air-PAW [10]. However, I.E. Vlad *et al* [19] stated that lower pH value was inconsequential to the inhibition of bacteria. He-PAW constituted optimum concentration of H_2O_2 to inactivate the bacteria but it was less efficient than air-PAW [10] and this inactivation was possible by inducing changes in osmotic pressure of cells [19].

2.6 Oxygen

O_2-PAW showed higher concentration of H_2O_2 when compared with the other PAWs (prepared in atmosphere of air and nitrogen) while the NO_3^- and NO_2^- were confined to be lower. When Renwu Zhou *et al* used oxygen as the working gas, it displayed 90.92ppm and 51.69ppm concentration of NO_2 and O_3 whereas NO concentration was found to be negligible. It improved the seed germination by 11.67%. The plant length dramatically increased with increase in incubation time to 7 days. O_2-PAW helped in chapping of seed, which improved its ability to absorb water and nutrients. It also contributed to decrease the MDA content in plants and enhanced the Indole Acetic Acid percentage in plants [10].

Ruonan Ma *et al* prepared PAW with argon mixed with oxygen as the feed gas, the O_2 present in it gave rise to atomic oxygen which was responsible for the formation of other ROS such as hydroxyl radical, singlet oxygen, superoxide anion and H_2O_2 [12].

Amalia Azzariti *et al* stated that ROS supports to bring about a significant effect against cancer cells. [21] investigated impact of O_2 plasma activated liquid medium on cancer cells (Hmel1, HBL and PANC-1) for 4, 24, 48 and 72 hours respectively. Higher amounts of ATP released and the cell showed much more CRT (Calreticulin) exposure for 72 hours treatment of plasma activated liquid medium. O_2-plasma activated medium induced apoptosis which caused characteristic cell changes and death and it also acted as immunological cell death inducer [20].

2.7 Carbon Dioxide

The content of acid formed in PAW obtained by J Julák *et al* was determined to be negligible when CO_2 was the working gas and the pH value remained unchanged even after 1 year of storage. It preserved its activity up to highest dilutions since it had greater concentration of H_2O_2 and that could have been due to higher efficiency of positive DC discharge. The acidity of this water was attributed to the formation of carbonic acid produced in it [5]. Toshihiro *et al* observed that PAW obtained through carbon dioxide gas produced large amount of singlet oxygen and the reactions are as follows:

$$O_2 + e \rightarrow 2O + e$$

$$CO_2 + e \rightarrow CO + O + e$$
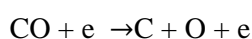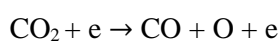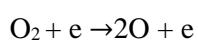
$$CO + e \rightarrow C + O + e$$

Table 1 summarizes the chemical properties of PAW formed in different atmospheres. (Values in the table are approximate and depend on the type of equipment used, liquid sample and activation time)

Table 1: *The chemical properties of PAW formed in different atmospheres*

Working Gas	pH Values	Hydrogen peroxide (μM)	Nitrites (μM)	Nitrates (μM)	References
Air	2-7	58-4117.89	173-1200	967-5274	N.Punith *et al* [4], J Julák *et al* [5], Renwu Zhou *et al* [10].
Ammonia	7.68-9.18	117.64	176.4		Pradeep Lamichhane *et al* [7]
Argon	2.19-3.5	227-1190	29-900	94.5	J Julák *et al* [5], Ruonan Ma *et al* [12], Konstantin Artem'ev et al. [13].
Nitrogen	3.4-5.8	53-220	20-1500	100-7919	J Julák *et al* [5], Priyanka Shaw *et al* [15], Chae Bok Lee *et al* [16], Joanna Pawlat *et al* [17].
Helium	1.79-7.5	160-910	18.3-30	2.7-470	Renwu Zhou *et al* [10], S. Zhang *et al* [18], I.E. Vlad *et al* [19].
Oxygen	4.1-7.4	63-1760	1.8-50	62-130	Renwu Zhou et al, [10], Ruonan Ma *et al* [12].

3   Future Application

As it is known that PAW efficiently inactivates bacteria and bacteriophages by causing damage to biological macromolecules, but its effect on COVID-19 has been reported yet. A study on pseudoviruses with the SARS-CoV-2S protein and it was noted that PAW effectively inhibited the pseudoviruses through S protein inactivation [22].

4   Conclusion

PAW can be prepared using various feed gases such as air, oxygen, nitrogen, argon, helium, ammonia and carbon dioxide. The outcome provided by PAW using various mediums as discussed were mainly related to higher yield, seed germination, better plant development, less prone to microbial growth in the crops, anti-carcinogenic action and decontamination of surfaces. The applications of PAW prepared in various gases had their own significance as they were found to possess unique properties in different mediums. One of the main applications of the plasma-activated water is that it can be used as an alternative for conventional nitrogen fertilizers. The air-PAW has the potential to increase the yield of plants than other PAWs and can also inactivate microbial growth. While O_2 plasma-activated medium induced apoptosis which caused characteristic cell changes and death and it also acted as immunological cell death inducer. Also, Ar, He PAWs displayed higher contents of ROS which contribute to inactivation of bacteria. Hence, it can be used as an alternative for insecticides and pesticides. It was noted that the conductivity of water increased with plasma treatment in N_2. Implementation of PAW is quite simple and has many applications in agriculture and medicine. PAW prepared by high voltage dielectric barrier discharge system could inactivate *Staphylococcus aureus* and *Escherichia coli* and retained efficacy even after 18 months.

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5   Declarations

5.1   Acknowledgments

5.2   Competing Interests

The authors declared that they do not have any conflict of interest in publishing this work.

How to Cite this Article

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