Characterization of Plasma Active Water and Its Sterilization Process Study

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Abstract. Plasma active water (PAW) has owned a good bacteriostatic performance, which can effectively clean the harmful microorganisms from the surface of fruits and vegetables, and has a wide application in the field of food cold sterilization. Gliding arc discharge was adopted in this study to form PAW liquids, with *Escherichia coli* (*E. coli*) as treatment object, and the sterilization process and the characteristics of PAW was optimized and measured through the determination of hydroxyl free radicals, pH, ORP, conductivity, showing that PAW was a green and residue-free sterilization technology. The experimental results showed that PAW prepared by air discharge had the best sterilization effect, followed by oxygen and nitrogen. PAW through air plasma treatment has a higher ORP value and longer efficiency, which was consistent with the results of pH and conductivity. According to Fenton reaction, PAW contained high concentrations of hydroxyl radical, which was responsible for its good bactericidal effect.

Keywords: Low temperature plasma activated water · Glide arc discharge · Sterilization effect · Fenton reaction

1 Introduction

Plasma is one kind of discharged gas realized in the atmosphere or vacuum state, which is the fourth state of matter, accounting for 99% of the space of the universe, and widely exists in the natural environment, such as Aurora, Mars, Sun and Thunder and Lightning [1]. Atmospheric plasma is a low-temperature plasma. The active substances produced innerside deionized gas contain electronic, excited molecules, high-energy particles, atoms, reactive oxygen species, active nitrogen and other active ingredients, which have bactericidal effect on bacteria, spores, viruses and fungi [2–5]. Plasma active water (PAW) is a liquid containing some active components of plasma under atmospheric plasma treatment by exposing the liquid to various forms of plasma discharge [6]. Plasma active water can clean various surfaces of food, fresh produce, and so on, and provides an indirect treatment for the storage and fresh preservation in food and agriculture area.

In recent years, PAW has shown many characteristics, such as high efficiency, simple and portable, safety and effect of killing a variety of food borne pathogenic bacteria [7]. Which exists great potential to replace traditional food sterilization

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technology and auxiliary. Along with the deepening of the research on the PAW sterilization, sterilization effect of PAW is generally regarded as a good cold sterilization technology due to low temperature (room temperature) processing, no residue, low energy consumption, simple operation, strong sterilization ability and other characteristics, very suitable for processing of heat sensitive material surface. [8]. However, based on the literature research, PAW is a novel sterilization method of low-temperature plasma sterilization, whose sterilization function and sterilization mechanism are still in the preliminary stage and needed to be further studied.

Compared with other low-temperature plasma discharge forms, gliding arc discharge technology, gliding arc discharge can be formed directed through the two naked electrodes, and has strong bactericidal ability, low energy consumption and high efficiency for the potential of industrial application [9]. For example, Zhang et al. studied the treatment of fresh radish with gliding arc discharge, and found that the bactericidal rate of gliding arc discharge reached 92.35% at 170 V within 5 min [10]. This study would adopt gliding arc discharge technology to prepare PAW, investigate the bactericidal effect of PAW, and analyze its internal active components, in order to further reveal its bactericidal mechanism.

2 Materials and Method

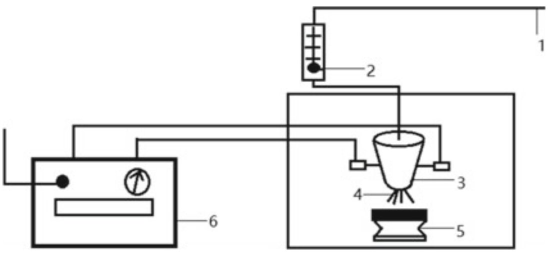
2.1 *Escherichia coli* Inoculation

Escherichia coli (*E. coli* ATCC8099) is provided by the China Industrial Microbial Species Management Center. *Escherichia coli* was incubated in a 37 °C and 180 r/min for 12 h, and then mixed with the prepared PAW. After the sterilization treatment of 5–10 min, 1.0 ml of the mixed liquid was extracted into counting medium of total number of colonies, and then cultured in constant temperature horizontal wet box for 24–48 h until the number of colonies can be counted according to the plate count method.

2.2 Preparation Instruments of PAW

The atmospheric plasma discharge processor was mainly composed of a power source, a processing chamber, a gas flow test table. There were two parallel copper electrodes in the reaction chamber (length and diameter size of 93 mm and 3.0 mm, respectively). Working gases, such as Air, O_2, N_2, were introduced from the top of the reactor, and ionized by a voltage of 10 kV between the two copper sheets when passing through the reactor. The samples were placed at the bottom part of the reactor on the sample table (Fig. 1).

Once PAW was prepared, *E. coli* suspension was placed in the plasma activated water with different ratios of 1:1 for 5 min, then adopted 1 ml of mixed liquid into the culture dish, each group is three parallel, and was incubated for 24–48 h in a constant temperature and humidity chamber.



Note :1. Gas source 2. Gas flow meter 3. Reactor
4. Slide arc discharge 5.Sample table 6.power supply

Fig. 1. Atmospheric plasma discharge equipment

2.3 Methods

2.3.1 Determination of ORP Value of Plasma Activated Water

150 ml of sterile deionized water in a 200 ml beaker was treated under plasma equipment with different discharge gases (Air, N_2, O_2) for 1 min, 2 min, 3 min, 4 min, 5 min. Once prepared, PAW was immediately measured using a HQ30d ORP tester, with three replicates in each set, deionized water as a control.

2.3.2 Determination of Hydroxyl Radical Concentration

Using Fenton reaction principle, through hydrogen peroxide reaction to formhydroxyl radicals, and 2,3-dihydroxybenzoic acid formed by the reaction of hydroxyl radicals with salicylic acid can be detected at 510 nm by visible light spectrophotometer (TU-1810, Shanghai Shengke Instrument Equipment Limited), When the salicylic acid added to the solution was fixed, the measured trend of the change of 2,3-dihydroxybenzoic acid and hydroxyl radical was positively correlated.

20 ml of prepared plasma activated water was Pipetted into the culture dish, numbered as 1, 2, 3, 4, 5, 6. After treatment, 13 ml was chosen into a 20 ml centrifuge tube and added 1 ml of iron sulphate solution and 1 ml of salicylic acid. All the treated samples were uniformly subjected to a water bath for 15 min, and the samples were taken at a wavelength of 510 nm by an visible light spectrophotometer, where the absorbance value represented the intensity of 2,3-dihydroxybenzoic acid content.

2.3.3 Determination of pH Value

For the determination of pH value, 2 ml of the bacterial suspension was transferred to 18 ml of distilled water, and the pH was measured by pH meter (PHB-4 Shanghai Yidian Scientific Instrument Company limited). Each treatment group was repeated three times.

2.3.4 Conductivity Measurement of PAW

Conductivity is an important parameter affecting the efficiency of discharge deactivation [11]. The conductivity of bacterial liquid and distilled water was determined in this part of the experiment. 2 ml of the bacterial suspension was mixed into 18 ml of distilled water, and measured by conductivity meter (DDS-11A, Shanghai Yidian Scientific Instrument Company limited).

3 Results and Discussions

3.1 Sterilization Effect of *E. Coli*

PAW formed under different gas discharge radiations, has different bactericidal effects due to different active ingredients. In this study, atmospheric plasma formed through air, nitrogen and oxygen discharges, was used to prepare PAW for *E. coli* inactivation treatment, as shown in Fig. 2. It can be seen that total number of colonies decreased with plasma radiation time, indicating a good sterilization effects. Among air, oxygen and nitrogen of discharge gas species, the sterilization effect of oxygen was the least obvious. When the treatment time was 0–2 min, the sterilization ability of PAW for oxygen discharge gradually increased, and the best sterilization effect was achieved at 2 min, and then remained unchanged. The bactericidal effect of air and nitrogen discharge is obviously better than that of oxygen. After nitrogen discharge treatment for 4 min, total number of colonies decreased to 24 CFU/ml in comparison with 325 CFU/ml of control samples. After 4 min of sterilization the total bacterial colony number reached 0 CFU/ml with obvious bactericidal effect for air discharge, reaching the aseptic state after 5 min treatment. It can be deduced that, if the discharge gas was selected properly, gliding arc discharge technology can completely inactivate *E. coli* within a short time duration of 5 min, indicating a good bactericidal effect. Tian et al. [11] has adopted a direct current atmospheric pressure cold plasma microjet to produce plasma activated water, and found that PAW exhibited stronger disinfection efficiency, which was associated with ORP and conductivity, since the cell membrane integrity and membrane potential of Staphylococcus aureus were destroyed more severely. Shen et al. [12] has adopted argon plasma discharge to inactivate *Staphylococcus aureus* suspended in the liquid, and found that direct plasma treatment for 40 min results in more than 2.0-log cell reduction, which can be contributed to the presence of the presence of hydroxyl radicals and atomic oxygen from the plasma and water interaction. Therefore, it can be deduce that the inactivation efficient would have a close relationship with the characterizes of PAW, as will be further revealed as follows.

3.2 Characteristics of PAW Under Different Discharge Gas Species

3.2.1 Conductivity and ORP Evaluation of PAW

According to Nernst equation and electrochemical reaction kinetics, the essential process of plasma and water interaction was a complicatedly redox process. In this study, OPR value was firstly used to test the redox properties of PAW. Figure 3 showed the change of ORP value with the treatment time in different discharge gases. It can be seen that ORP value of PAW prepared by air, nitrogen and oxygen plasma discharge increased with plasma radiation time. Initial ORP value of deionized water was obtained as 379.7 mV, through different gas plasma discharge of air, nitrogen and oxygen, ORP value reached ultimately 558.0 mV, 489.1 and 452.9 mV, respectively,

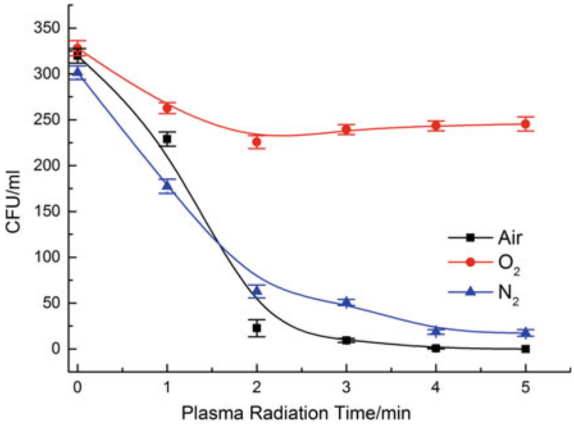


Fig. 2. The sterilization effects of PAW on *E. coli* under different discharge gas species

indicating a strong oxidizing effect. ORP from air discharge was the highest, followed by nitrogen discharge, and oxygen discharge water was the lowest, which is much more than that of direct current atmospheric pressure cold plasma microjet results [13].

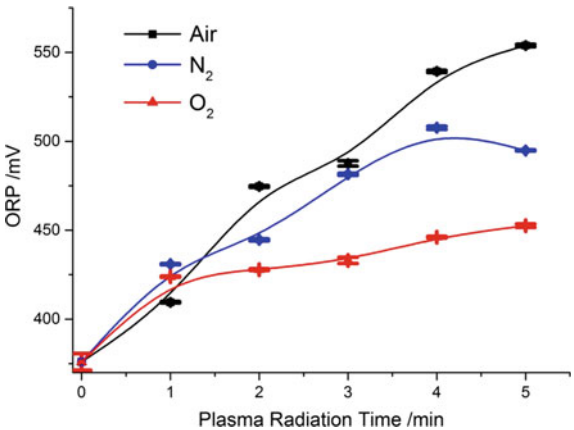


Fig. 3. The change of ORP value under air, N_2 and O_2 plasma activated water under different radiation time

Conductivity of treated liquids reflected the ion concentration and the ability of a solution to conduct a current. The more charged ions in a solution, the greater the conductivity. Figure 4 showed the trend of PAW conductivity variation with discharge power under different gas discharge irradiation. It could be seen that the conductivity of PAW increased with plasma discharge power, during which the conductivity of PAW prepared under air plasma radiation was higher than nitrogen, and oxygen was the lowest. Xin Qing [14] et al. found that the increase of solution conductivity was resulted from the increment of the concentration of active ions contained in the liquid. With the increment of plasma discharge power, the ion and electron concentration generated by gas ionization increased, leading to the higher conductivity of PAW.

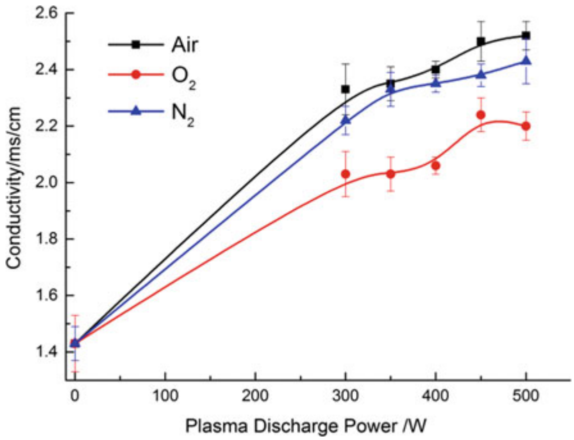


Fig. 4. Effect of plasma discharge power on the conductivity of PAW under different gas species

3.2.2 Evaluation of pH and Hydroxide of PAW

Figure 5 showed the trend of pH value of PAW under different gas species. As can be seen, the treated water was transferred into acidic liquid after plasma treatment, which had a positive effect on the survival of bacteria. Oxygen plasma has the lowest pH decrement, followed by nitrogen, and air plasma has the largest decrement. Regardless of discharge gas species, when plasma radiation time was kept for 1 min treatment, the pH value dropped rapidly to about 3.5, and then changed gradually down to 3.0 with the treatment time. In fact, this lower pH value was may be due to the interaction and diffusion effect between gliding arc discharge and deionized water surface, since Satoshi et al. has used atmospheric pressure plasmas treating the surface of an aqueous solution to inactivate bacteria suspended in the solution, and found that critical pH value may arise from the equilibrium reaction between O_2 and hydroperoxy radicals HOO, which is known to be approximately 4.8 [15].

Figure 6 showed the variation trend of the hydroxide absorption intensity of deionized water treated by gliding arc discharge at different times according to Fenton reaction. As can be seen, the absorption intensity increased gradually with the extension of plasma radiation time. Nitrogen plasma treatment had the largest change in light absorption intensity, followed by air plasma treatment, while oxygen treatment had the smallest influence on light absorption intensity. With the extension of plasma radiation time, both nitrogen and air plasma had a linearly increment, while oxygen plasma had a smaller rise. In addition to hydroxyl radicals, PAW may also contained other active bacteriostatic ingredients, such as nitrite ion and nitration, ozone, etc., which contributed the larger rise of nitrogen or air plasma and would be further verified in future studies [16].

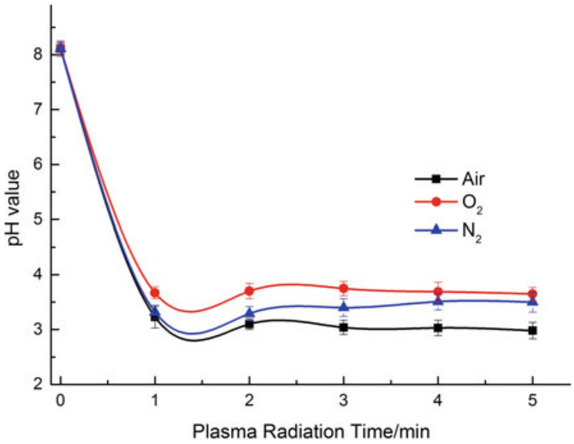


Fig. 5. Effect of plasma radiation time on pH value of PAW under different gas species

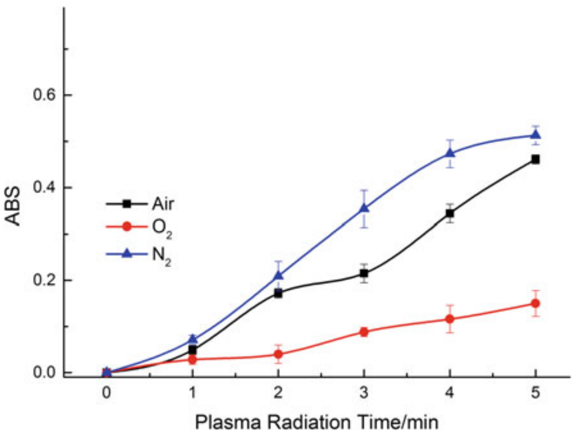


Fig. 6. Effect of plasma radiation time on hydroxide absorption of PAW under different gas

3.3 Timeliness of Plasma Active Water

Once the plasma is prepared, can its bacteriostatic properties be preserved permanently and thus have a long-term bactericidal effect? This study studied the timeliness of PAW from the respective of ORP variations with storage time. Figure 7 showed ORP variations of PAW under air discharge as a function of storage duration, since strong oxidizing (high ORP value) could damage cell membrane and thus lead to bacterial oxidative damage [11]. Therefore, ORP could be chosen as indicator for sterilization ability of PAW. ORP value of PAW was initially 548.5 mV, showing the redox potential was still very high within first 1–2 h with storage time until ORP value was fed back to the initial state of 350 mV after 5 h. However, ORP value of PAW prepared by air discharge does not change much while that prepared by nitrogen discharge and oxygen has a similar decreasing trend. It can be concluded that the ORP value of the activated water in the air discharge plasma is higher than that in both the nitrogen and oxygen discharge, which may be caused by more gas components or active substances and indicating a strong timeliness of PAW more than 5 h.

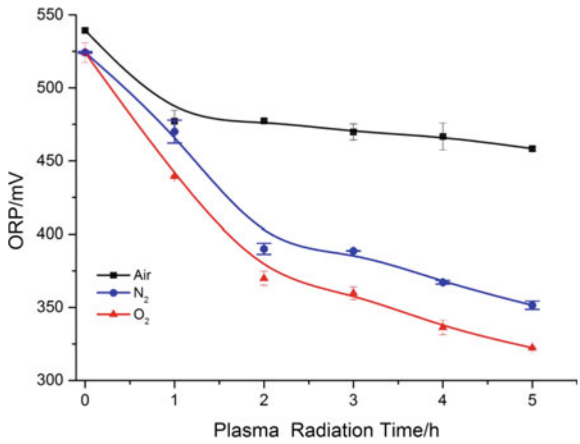


Fig. 7. ORP variations of PAW under air discharge as a function of storage duration

4 Conclusions

In this study, PAW has been manufactured by gliding arc discharge under different discharge gas species with *E. coli* as treatment object. Sterilization effects showed that bactericidal effect of PAW prepared through air and nitrogen discharge plasma is obviously better than that of oxygen, where the total colony number reached 0 CFU/ml after 4 min plasma radiation duration. The excellent inactivation efficient would have a close relationship with the inner side components of PAW, as was revealed by measuring the characteristics of PAW, such as hydroxyl free radicals, pH, ORP, conductivity. The characterization of PAW showed that the activated water after air plasma irradiation has a higher ORP value and a longer time efficiency, which was consistent with the test results of pH and conductivity. According to reaction, the plasma active water under air plasma may contain high concentration of hydroxyl radicals and other active ingredients, which has a good bactericidal effect, indicating that PAW was a green and residue—free sterilization technology.

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