# Low-Temperature Air Plasma Jet for Inactivation of Bacteria (*S. Aureus* and *E. Coli*) and Fungi (*C. Albicans* and *T. Rubrum*)

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Generally, most of the setups of plasma jet adopted rare gases (e.g., helium and argon) as the working gas. The drawback of using rare gases is that it cannot be continuously used as the gas tank volume is limited, and the cost of using rare gases to yield plasma jet is also higher. Thus, a plasma jet using solely air as the working gas could be a potentially promising solution. In this study, a low-temperature air plasma jet using only air as gas was developed. The device was optimized by adjusting the control circuit (deadband, current, voltage and duty ratio), frequencies (50–350 kHz), air pump flow-rate (0–8 L/min), and geometric size of each component. The spectrum of the low-temperature air plasma jet revealed that the main components are $N_2$, and the radicals N/NO occupy 92.5%, while O and $O^+$ occupy less than 7.5%. Under the optimal conditions, where the discharging frequency was at 120 kHz and the output voltage was set to 5–10 kV, its inactivation ability toward microorganisms, such as *Staphylococcus aureus*, *Escherichia coli*, *Trichophyton rubrum*, and *Candida albicans* was assessed. In addition, the inactivation ability of low-temperature air plasma jet and ozone toward microorganisms was compared. Low-temperature air plasma jet and ozone were applied to microorganisms for a range of treatment times, and the results showed different degrees of microbiological inactivation. The inhibition zones formed on the agar plate were 3.8 cm (*Staphylococcus aureus*), 3.3 cm (*Escherichia coli*), 5.3 cm (*Trichophyton rubrum*), and 3.2 cm (*Candida albicans*) when a 15 min low-temperature air plasma jet was applied. Comparatively, the diameters of the inhibition zone after 15 min of ozone exposure were 3.8, 5.0, 0.9, and 0.0 cm, respectively. Collectively, the low-temperature air plasma jet showed comparable efficacy to ozone in terms of inactivation ability. This low-temperature air plasma jet demonstrated its potential to be used for medical applications involving the inactivation of microorganisms.

topics: low-temperature air plasma jet, ozone, microorganism, inactivation

## 1. Introduction

Over the past decade, the technology of atmospheric pressure non-equilibrium plasma jet has become one of the important research fields in academia and medicine [1]. This technology applies plasma to sterilize surfaces or to inactivate microorganisms. In general, a plasma jet contains reactive electrons, ions, atoms, molecules and other substances, as well as active particles such as free radicals with certain oxidation activity [2]. To trigger the plasma jet, the current conventional discharging geometry structure is mainly a plate-to-plate configuration or a coaxial cylinder configuration. The plate-to-plate configuration is easier to make, but to form the plasma jet, an extra jet flow guide must be added. The coaxial cylinder configuration has an advantage over the prior in producing the plasma jets, yet the uniformity of the discharging depends on the axial symmetry of the device.

Almost all conventional setups of plasma jet have adopted rare gases, such as helium and argon, as working gas to produce a plasma jet for medical purposes [3]. Only a couple of cases employed rare gases mixed with $O_2$ or $N_2$ or certain percentage (usually below 5%) of air. Using rare gases to stimulate a plasma jet for medical purposes leads to a waste of resources. In addition, the application of rare gases plasma jet is also restricted and unsustainable as a gas tank is required. Also, the cost of this type of plasma jet is higher. In order to overcome these problems, jet plasma using solely air as the working gas could potentially be the solution. Within this project, a handheld device, the so-called low-temperature air plasma jet (LTAPJ), was built and used to explore the sterilization effect against different microorganisms. This LTAPJ device produces a plasma jet with a considerably high concentration of radical particles mixed with medical standard ozone concentration. The prototype design and optimization of this device have been further discussed. Besides, in order to verify that LTAPJ can effectively kill bacteria and fungus, a comparison was carried out on four strains by the traditional ozone treatment.

## 2. Materials and methods

### 2.1. Optimization of the prototype design of low-temperature air plasma jet (LTAPJ)

The LTAPJ used in this project was designed by Suzhou Amazing Grace Medical Equipment Co., Ltd. (Suzhou, Jiangsu Province, China). This device was patented in 2020 (patent No. 201920229222.9). The optimization of LTAPJ for parameters such as control circuit (deadband set to 0.88 $\mu$ s, current to 2.08 A with input voltage of 12.8 V, duty ratio chosen to be 50%), frequencies (confined to 120 kHz), air pump flow rate (set to 5.0 L/min) and geometric size of each component was specified by carrying out many tests. To optimize these parameters, an enumeration method was adopted to find the best plasma jet parameters suitable for this study, i.e., to choose the best deadband value. All possible values were tested and among these values, 8.80 $\mu$s was the best condition when the current shown to be at its minimum, indicating the lowest ion production, which also means that the temperature of the plasma jet met the lowest value for the LTAPJ setup.

### 2.2. Bacterial and fungal culture

Four types of microorganisms were selected for culture sterilization experiments: *Staphylococcus aureus* ATCC 6538 (SA), *Escherichia coli* ATCC 25922 (EC), *Candida albicans* LM186382 (CA) and *Trichophyton rubrum* ATCC 28188 (TR). The bacteria were purchased from Qingdao Hope Bio-Technology Co., Ltd., China, and the fungi were purchased from LMAI Bio, China. In brief, *S. aureus* and *E. coli* were cultured on Tryptic Soy Agar (TSA) at 37°C for 24 h; whereas *C. albicans* and *T. rubrum* were cultured on Potato Dextrose Agar (PDA) at 27°C for 48–72 h. Upon colony formation, the loopful of bacterial or fungal culture was transferred into a 250 mL Erlenmeyer flask containing 50 mL of Tryptic Soy Broth (TSB, for bacteria) or Potato Dextrose Broth (PDB, for fungi) for suspension culture preparation. The flask was then incubated in the appropriate temperatures with constant shaking of 200 rpm for 24 h (bacteria) or 48–72 h (fungi). The suspension culture was used for plate spreading in the following experiments.

### 2.3. LTAPJ treatment of bacteria and fungus cultured on agar

According to the cell turbidity (based on optical density $OD_{600\,nm}$) as measured in the spectrophotometer, the bacterial or fungal suspension culture was adjusted till $OD_{600\,nm} = 0.5$. Then, 0.1 mL of the diluted culture was spread onto a fresh TSA or PDA plate. Prior to incubation, the plate was subjected to LTAPJ treatment. Considering that the inactivation effect of LTAPJ on microorganisms is related to factors such as the distance between the nozzle of the experimental device and the surface of the bacterial species, as well as the plasma gas composition and the flow rate of the discharging gas [4], we chose the distance between the strain and the plasma jet at 2 cm during the experiment, and the plasma jet flow rate was maintained at 4 L/min. The discharging frequency of LTAPJ was 120 kHz, and the voltage connected to the discharge pole was set to 5000 V (±5%). The LTAPJ treatment time ranged from 0–15 min, and the treated plates were placed in an incubator for culture growth. The zone of inhibition size was recorded after 24–72 h of incubation. A culture plate without LTAPJ treatment was used as a control setting.

### 2.4. Ozone treatment of bacteria and fungus cultured on agar

Similar to above (Sect. 2.3), the culture was spread onto a fresh agar plate and subjected to ozone treatment afterward. The spread plate was placed right beneath the ozone source (100 mg/h) with a time ranging from 0–15 min. Then the ozone-treated plate was incubated for 24–72 h for the purpose of inhibition ozone observation. A plate culture without ozone treatment was used as a control group.

## 3. Results and discussion

### 3.1. Optimization of the prototype design of low-temperature air plasma jet (LTAPJ)

The device named AGPPC, as shown in Fig. 1, is constituted of discharging part, control circuit, air pump, plasma generating circuit etc. The device was successfully optimized by adjusting the control circuit (deadband, current, voltage, and duty ratio), frequencies (50–350 kHz), air pump

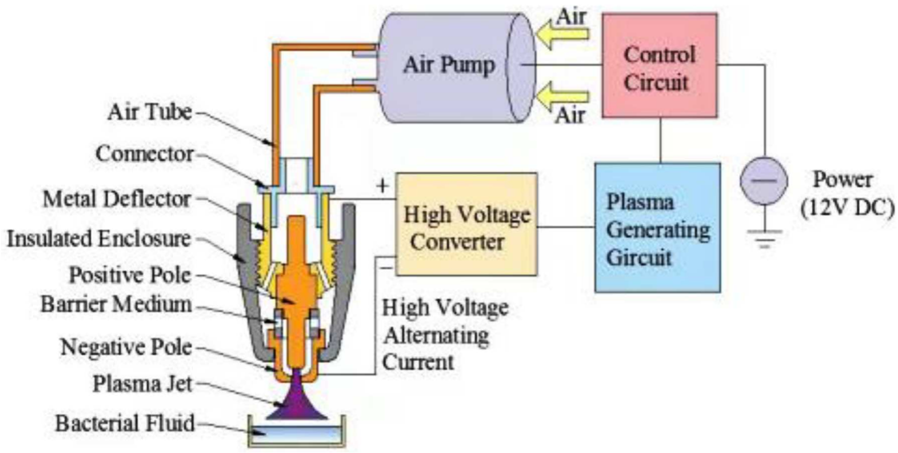


Fig. 1. Section view of LTAPJ discharging structure of this study.

flow rate (0–8 L/min), and geometric size of each component. The final experimental trial of the optimization process with a fixed frequency of 120 kHz and a fixed duty ratio of 50% was shown in Table I. With a fixed frequency and duty ratio, by choosing a different deadband range, the discharging states can be distinguished into stable or unstable conditions. Meanwhile, under stable conditions, when the deadband was chosen to be E9, which represents a time 8.80 µ s with an input voltage 12.8 V, then the current in the circuit gained the minimum value of 2.08 A, which signifies the lowest discharging strength (ion concentration) of the device. Through this adjusting method, we were able to find out the minimum value of the ion concentration leading to the lowest temperature of 35°C.

AGPPC is different from the conventional design of plasma discharging structure as shown in Fig. 1. The unique structure of AGPPC (CE certificate GTS verification No. CLJS20110527615) is formed from an air tube, a metal deflector, an insulated enclosure, positive and negative poles, and a barrier medium. This structure, which solely uses air as the working gas, enables air to be pumped smoothly through the channels, and the air was sufficiently ionized. Meanwhile, the design of this structure helps to cool down the discharging part and makes the lifespan of this part longer. Also, thanks to the use of a deflector, the gas flowing through the channel has a larger contact area per unit time. This means that the air can be ionized with more opportunities, namely higher ionization efficiency.

Furthermore, usually most of the researchers apply the discharging frequency below 50 kHz (see Table II and [5–10]). When the frequency of the discharging circuit is higher, the design of the generating and controlling circuits is more difficult as it brings in power dissipation, chip cooling, impedance matching and etc. This makes it more difficult to design the whole plasma-generating structure. Our current work overcomes these challenges by setting the deadband to 8.80 $\mu$s, the current is then measured as 2.08 A and the voltage as 12.8 V with 50% of a fixed duty ratio at a frequency 120 kHz. Under this condition, the amount of ozone was reduced to 0.12 ppm, which was under the industry hygiene standards (0.15 ppm) of ozone usage. This concentration makes it unnecessary to worry about the harm caused by excessive ozone concentration in the actual medical field, especially where human mucosal tissues are exposed to plasma jet.

TABLE I

The final experimental trial of optimization processing of LTAPJ.

| Deadband range [$\mu$s] | Current [A] | Voltage [V] | Stability | Plasma dose [J] |
|---|---|---|---|---|
| 0.72 | 5.00 | 13.0 | ✓ | 26.0 |
| 2.66 | 2.40 | 13.0 | ✓ | 9.1 |
| 1.53 | 5.00 | 11.5 | ✓ | 23.0 |
| 0.90 | 5.00 | 13.7 | ✓ | 27.4 |
| 1.22 | 2.30 | 12.8 | ✓ | 8.3 |
| 1.40 | | | × | |
| 1.58 | 4.00 | 13.4 | ✓ | 20.1 |
| 1.71 | 3.00 | 13.0 | ✓ | 13.0 |
| 2.17 | 2.60 | 12.8 | ✓ | 10.2 |
| 2.75 | 2.34 | 12.8 | ✓ | 8.6 |
| 3.00 | 2.17 | 12.8 | ✓ | 7.5 |
| 3.11 | | | × | |
| 3.25 | 2.15 | 12.8 | ✓ | 7.4 |
| 8.80 | 2.08 | 12.8 | ✓ | 6.9 |
| 6.90 | | | × | |
| 2.44 | 2.60 | 12.8 | ✓ | 10.2 |

Note that the low-pressure discharge gas has a lower density, resulting in a lower collision frequency between electrons and neutral particles. Therefore, electrons easily gain higher energy under a high electric field. This facilitates the ionization

TABLE II

The comparison of different plasma jet structure.

| | Structure | Frequency [kHz] | Flow-rate [L/min] | Voltage [kV] | Ion form | Temperature [°C] | Working gas |
|---|---|---|---|---|---|---|---|
| Ning [5] | coaxial single electrode DBD | 25 | 0.4–2.8 | 3.5–4.5 | plasma jet | 50–200 | helium |
| Jacofsky et el. [6] | plate–plate DBD | 0.4 | 16.5 | 25 | plasma jet/plume | ambient | helium |
| Shao et al. [7] & Weng et al. [8] | capillary tube | $13.56 \times 10^3$ | 10.0 | 0.57 | micro plasma jet | 40 | $N_2$/Ar |
| Kim et al. [9] | hollow tube | 36 | $35 \times 10^{-3}$ | 6 | plasma plume | ambient | helium |
| Darmawati et al. [10] | coaxial electrode | 18.32 | 1.0 | 9.58 | plasma jet | ≈ 87 | Ar |
| AGPPC (this study) | irregular coaxial electrode | 90–150 | 0.5–5.0 | 5–10 | plasma jet | 35–45 | air |

process, making it easier to obtain higher-density plasma with low temperature [11]. According to this principle, the discharging structure adopted in this work used an air flow rate pumped into a discharging chamber surrounded by an insulated enclosure, and this flow rate was set to about 5 L/min to ensure a relatively lower density of the discharging gas.

As for the plasma dose of LTAPJ, we simply calculate the discharging power per unit time. For instance, when the whole current of the device is measured as 2.08 A, the corresponding current in the control circuit has been tested at the value of 1.0 A, thus the discharging power regardless of the control circuit is $1.08 \times 12.8 \times 50\% = 6.9$ W, getting the plasma dose of 6.9 J.

Plasma can be generated by a variety of methods, such as corona discharge, dielectric barrier discharge, arc discharge etc. [12]. Corona discharge typically takes place at a frequency between 5 kHz and 50 kHz through high voltage bridges over the positive and negative electrodes. Dielectric barrier discharge (DBD) is a special type of corona discharge where the positive and negative electrodes are separated by a dielectric medium whose lifetime is extended. The arc discharge is generated under extremely high voltage conditions that lead to a sudden spark and generated arc plasma which vaporizes the electrode material. Obviously, DBD has a privilege over other method generating plasma, concerning the electrode life. Therefore, in this study, we adopted a coaxial DBD structure, but between the cathode and the anode, partial insulation was adopted, which made the device different from the structures of most researchers. Common materials such as copper, platinum, silver, tungsten, and stainless steel were used as the discharge electrode. In this study, we used zirconium and hafnium alloys materials as the discharge electrode. These materials have made the anode discharge capability (electron emission capability) far higher than that of conventional electrode materials (copper, platinum, silver, tungsten, and stainless steel). This makes the discharge structure adopted in this study a great advantage in ion yield compared to the conventional structure.

Plasma can be produced with different gases including nitrogen, helium, argon, and air [13]. In most cases, rare gases such as helium, argon and neon are used as working gases. Some scholars employ $CO_2$, $N_2$, $O_2$, or a mixture of these three as working gases, however, very few researchers apply air as working gas. In this study, the plasma discharge structure involved direct use of air as the gas source for the discharge with a controlled flow rate of 5 L/min, discharge voltage of 5 kV, and employs 90–150 kHz as the plasma generate frequency. The part between the cathode and anode is separated by a ceramic material. The use of air as the working gas contributed to several advantages, such as (i) it is an environmentally friendly method; (ii) no cost is required for the working gas; (iii) no gas tank or carrier gas channel is required; (iv) it permits plasma equipment to be practical as a portable device.

### 3.2. Spectrum of low-temperature air plasma jet (LTAPJ)

While discharging the air to generate a plasma jet, the main components are observed by AvaSpec multi-channel spectrometer as shown in Fig. 2. The optical emission spectrometer (OES) measurements were taken at a distance of 20 mm under the nozzle where the plasma jet is ejected out, and the pick-to-pick voltage was around 9.8 kV. The spectrum showed emission of species such as $O^+$ lines (236.0, 246.9, 258.5, 296.0, 777.4, 845.0 nm), $N_2$ lines (316.0, 337.2, 357.7, 380.5 nm).

In our scheme, the main radicals are $N_2$, N/NO, O and $O^+$. It is known that the more $N_2$ radicals are present, the longer the plasma life, which helps to maintain a longer inactivation time under the same conditions. In contrast, to get a low-temperature plasma jet, typically like the Chatraie's method [14], helium gas as the working gas is used, while the radicals O, He, and OH occupy the minority. Suppose the amount of the ions production by the Chatraie method and ours is the same, obviously our scheme has much more $N_2$ and N/NO radicals, which indicates a strong inactivation effect on bacteria and fungi. One more key

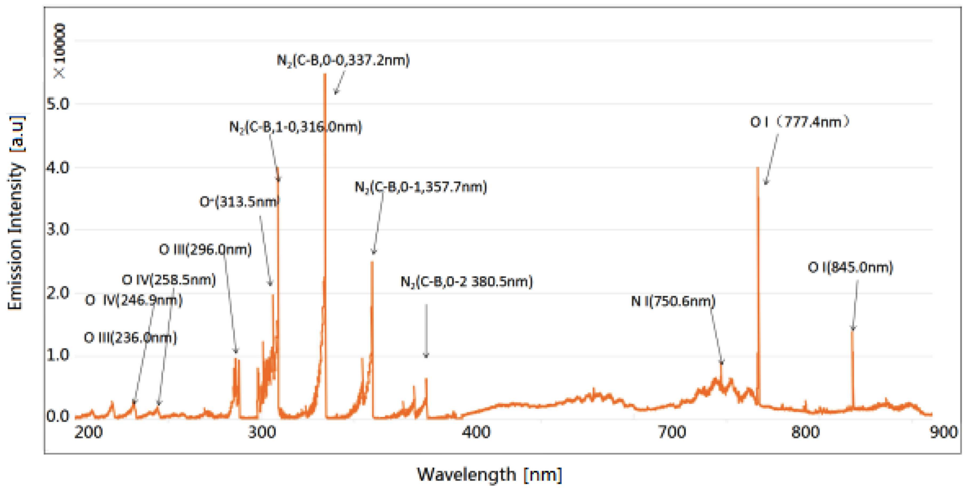


Fig. 2. Optical spectrum of LTAPJ.

point that should be noticed is that, in our scheme, the ozone production is 0.12 ppm, which meets the international medical standard (0.15 ppm), indicating that our technology is safe.

In our scheme also the air flow reduces the content of oxygen in the air gap and prolongs the life of the more metastable $N_2$ to the beginning of the next discharge, providing a large number of seed electrons necessary for the discharge. Thus, compared with the Chatraie's method, our scheme has clear advantages in ion yield. The radical particles obtained by our scheme are different from conventional methods. The spectrum of LTAPJ was analyzed using AvaSpec multi-channel spectrometer.

The key difference among the various structures for generating plasma is the form of the ions. Another feature is the working gas; researchers generally use rare gases, while in this work we employ air as the working gas, which makes the method more environmentally friendly.

### 3.3. LTAPJ and ozone treatments on bacteria and fungus

The developed LTAPJ device was used to assess its potential in microorganisms' inactivation. Figure 3a shows the results of LTAPJ treatment on different microorganisms with different treatment duration. Overall, the size of the inhibition zone was directly proportional to the duration of treatment; the longer the culture plate is exposed to LTAPJ, the larger the size of the inhibition zone. Besides, LTAPJ showed different inhibitory effect against different types of microorganisms. *C. albicans* required a longer treatment time as compared to the other three microorganisms to achieve a similar inhibitory effect. LTAPJ demonstrated highest inhibitory effect to TR (5.3 cm), followed by SA (3.8 cm), EC (3.3 cm), and CA (3.2 cm) after 15 min of treatment.

As mentioned earlier, we tested the composition of the plasma gas of LTAPJ with a fiber optic spectrometer (Fig. 2). The LTAPJ plasma gas consists mainly of reactive nitrogen species (RNS) and a minimum amount of reactive oxygen species (ROS). ROS has the capability to attack the polyunsaturated fatty acids of the cytoplasmic membrane and initiate a self-propagating chain reaction [15]. Nitrogen species likely interact with biological matter to generate further reactive species, producing nitrosative species that will be involved in protein degradation [16]. Besides, studies have shown that plasma contains free radicals, electrons, ions, high-speed particles and ultraviolet rays [17–20]. These different compositions can also damage cell membranes, DNA, and proteins in microorganisms, and even change the cytoskeleton structure to achieve a bactericidal effect [4, 21–24].

To give a better illustration on the disinfection capability of LTAPJ, a comparison was made by treating the microorganisms using 100 mg/h ozone on culture plates (Fig. 3b). The results showed that the microorganisms' inactivation via ozone treatment is also dependent on the treatment duration, which is similar to the findings obtained using LTAPJ. However, in terms of the effects on the microorganism types, ozone treatment shows the highest inhibitory effect to EC (5.0 cm), followed by SA (3.8 cm), TR (0.9 cm), and CA (0.0 cm) after 15 min of treatment. The antimicrobial effect of ozone is mainly due to the decomposing of ozone double bonds, which can destroy the plasma membrane of the cells [25]. Besides, ozone can achieve sterilization effect by attacking other cellular components such as proteins, unsaturated lipids, respiratory enzymes, and nucleic acids in the cells [26–29]. Although the use of ozone treatment at a concentration of 100 mg/h has showed inactivation ability toward microorganisms, this ozone concentration is considerably harmful to the human respiratory tract [30].

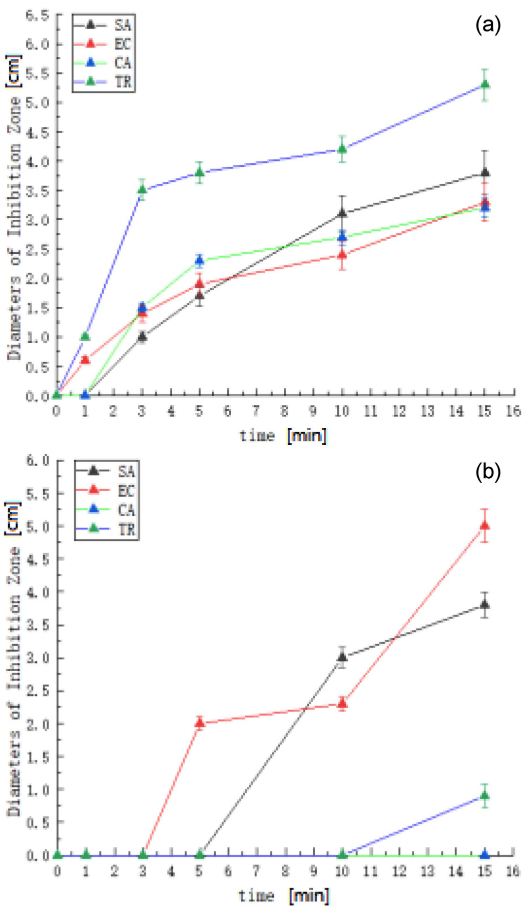


Fig. 3. Diameters of the inhibition zone forming on agar plate after treating with (a) LTAPJ and (b) ozone.

Collectively, both LTAPJ plasma and ozone worked similarly in attacking cellular structures such as the cell membrane, proteins, enzymes, and DNA in the cells. In this study, results showed that the plasma generated from in-house build LTAPJ device has similar disinfection ability as ozone with a production rate at 100 mg/h, whose ozone concentration is approximately 2–5 times higher than the international medical standard.

## 4. Conclusions

A low-temperature air plasma jet using solely air as the working gas has been developed and successfully optimized by adjusting the control circuit (deadband, current, voltage, and duty ratio), frequencies (50–350 kHz), air pump flow-rate (0–8 L/min), and geometric size of each component. The main radicals are $N_2$, N/NO, O and $O^+$ as observed in the optical spectrum. LTAPJ showed different inactivation effect against different types of microorganisms, with the highest inhibitory effect to TR (5.3 cm), followed by SA (3.8 cm), EC (3.3 cm), and CA (3.2 cm) after 15 min of treatment. LTAPJ and ozone demonstrated comparable inactivation ability toward microorganisms. This LTAPJ poses good potential to be used for medical application as well as epidemic diseases prevention.

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