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Sterilization of *E. coli* bacterium with an atmospheric pressure surface barrier discharge*

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The atmospheric pressure surface barrier discharge (APSBD) in air has been used in killing Escherichia coli (*E. coli*). There is almost no bacterial colony in the sample after treatment by discharge plasma for 2 min. A diagnostic technique based on mass spectrum has been applied to the discharge gas and the mechanism of killing is discussed. Ozone and monatomic oxide are considered to be the major antimicrobial active species. There is almost no harmful by-product. The experiment proves that APSBD plasma is a very simple, effective and innocuous tool for sterilization.

Keywords: surface barrier discharge, non-equilibrium plasma, sterilization
PACC: 5280, 5275R

1. Introduction

Atmospheric pressure discharges have been widely investigated in recent years [1−4] because they offer a variety of advantages, such as low cost equipment and discharge reactivity. A discharge generated at a dielectric surface can generate highly active non-equilibrium plasmas even at atmospheric pressure and room temperature. This type of discharge, called surface barrier discharge, is shown to be of great prospect for a number of industrial applications and environmental protections [5−8] and its characteristics have been studied.[9−11]

Several researchers have addressed the utilization of discharge plasma for the control of gaseous pollutants and the destruction of poisonous compounds, while the technology for biologic disinfection and sterilization still needs to be developed. Traditional methods such as ultraviolet radiation, high temperature and chemical suffocating have the shortcomings of halfway killing, degradation of medical instruments, leaving of dead angle, heavy workload and leaving of toxic by-products etc. New methods to sterilize at room temperature at low or atmospheric pressure are actively studied.[12,13] Gadri *et al*[13] used a one-atmosphere uniform glow discharge plasma in killing a variety of micro-organisms. In this work we use atmospheric pressure surface barrier discharge (APSBD) for sterilization of *Escherichia coli* (*E. coli*) bacteria. There are several merits of this kind of discharge plasma: the temperature is low, which avoids the shortcomings occurring in the high-temperature method; the plasma is innocuous and operators can stay near the discharge without the need of any precautionary measures; the process can be carried out in any conditions with only a portable high-voltage supply. We also discuss the mechanism of killing.

2. Experimental setup

Figure 1 schematically shows the experimental setup for the sterilization. A sinusoidal voltage up to several tens of kilovolt peak-to-peak was applied to the electrodes, and the source frequency was varied in the range of 1–20 kHz. Experiment was carried out at room-temperature in air, pectinate electrodes generated heat to some degree after 10 min, but the surface of *E. coli* sample which was 20 mm away from the plasma panel maintained room-temperature. The area of *E. coli* was about 28 cm^2 while the rectangular area of the pectinate electrode was about 9 cm^2. The current and voltage waveforms were recorded in a

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digital oscilloscope (Tektronics TDS220). The gas species were diagnosed by a mass spectrometer (LZL-203D).

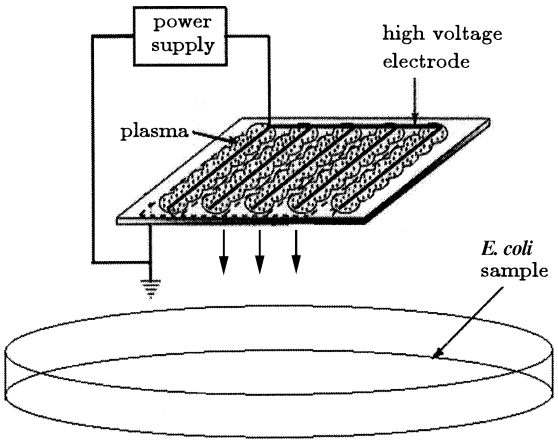
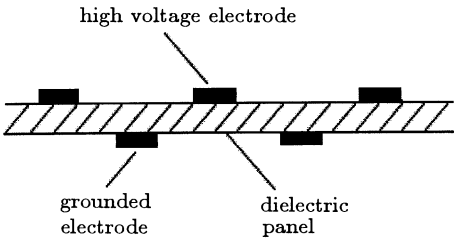
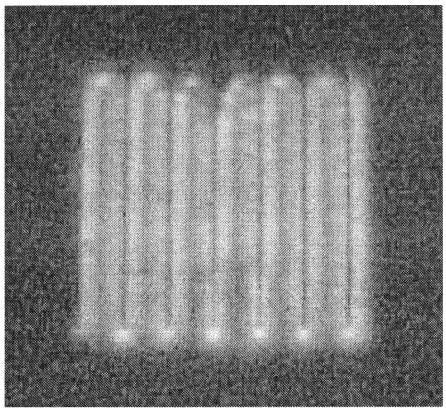


Fig.1. Experimental setup for sterilization.

The dielectric panels used is a 55 mm×55 mm copper-clad plate whose dielectric constant is 2.6; the thickness is 1.0 mm. Electrodes are made of red copper, covered with lead–tin foil. The high voltage electrode consists of seven interconnected electrode strips 1 mm-wide, 10 μm-thick, 33 mm-long with a strip-to-strip distance of 4 mm, and the grounded electrode consists of the same pectinate electrodes on the lower surface (Fig.2)



(a)



(b)

Fig.2. Schematic of dielectric panel and pectinate electrode (a), and image of discharge (b).

Figure 3 shows the typical voltage and current waveforms of APSBD. We can see that the current waveform consists of a sinusoidal wave with many current peaks. It indicates that this kind of discharge is not glow discharge, whose current is characterized by only one narrow peak every half cycle of the applied voltage,[2] but a dielectric barrier discharge (DBD).

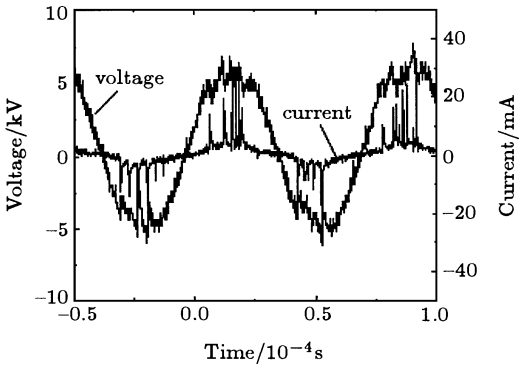


Fig.3. Typical voltage and current waveforms of APSBD.

3. Results and discussion

Figure 4 shows a survival curve of *E. coli* treated by the discharge plasma when the voltage peak to peak of 40 kV is applied and the treatment durations are 30 s, 1, 2, 5 and 10 min respectively. We can see that APSBD is very effective for sterilization: at the beginning, 0–2 min, the curve drops rapidly and a quick killing process is going along. The curve drops slowly after 2 min.

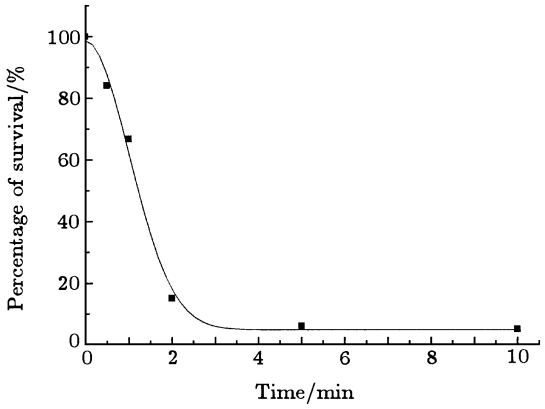


Fig.4. Survival curve of *E. coli*; the sample was exposed directly to APSBD plasma in air.

Figure 5 illustrates the photos of samples of *E. coli* that were incubated in an incubator for 14 h. One of them is the sample that had not been treated by

APSBD plasma, while the other five samples were treated for 30 s, 1, 2, 5 and 10 min respectively. We can see that there is almost no bacterial colony in the sample after being treated for 2 min.

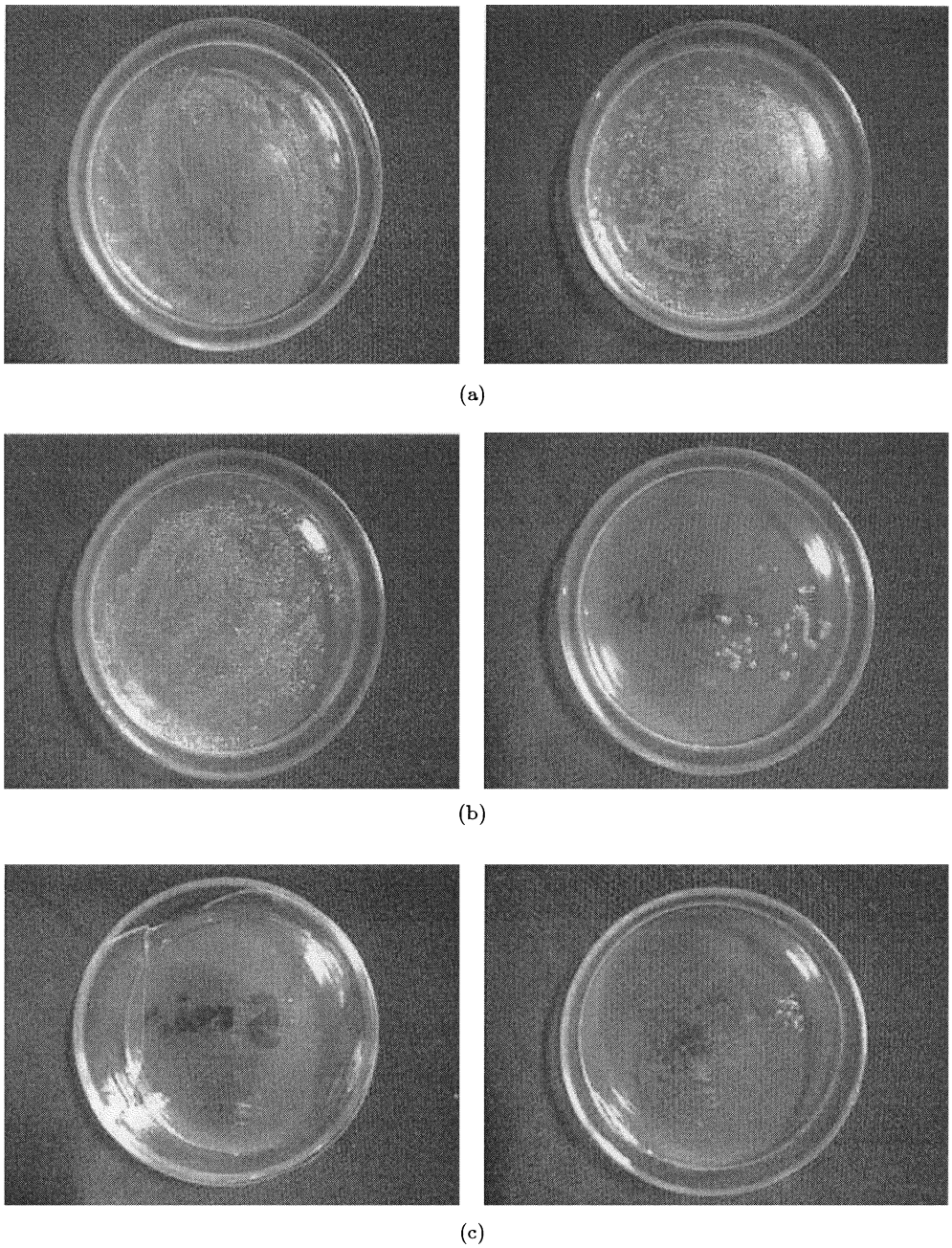


Fig.5. Samples of *E. coli* incubated in an incubator after treatment by air plasma for different durations. (a) Untreated (left) and treated for 30 s (right), (b) treated for 1 min (left) and treated for 2 min (right), (c) treated for 5 min (left) and treated for 10 min (right).

The mechanism of sterilization was one important thing we are concerned with. In order to know which species plays the major role in this process, we used a mass spectrometer to investigate the discharge plasma. The result is that molecular oxygen and monatomic oxygen increase with time, nitric oxide NO also increases but its content is much smaller compared with molecular oxygen and monatomic oxygen (Fig.6), while other species, such as NO_2, OH, HO_2, H_2O_2, have not been found.

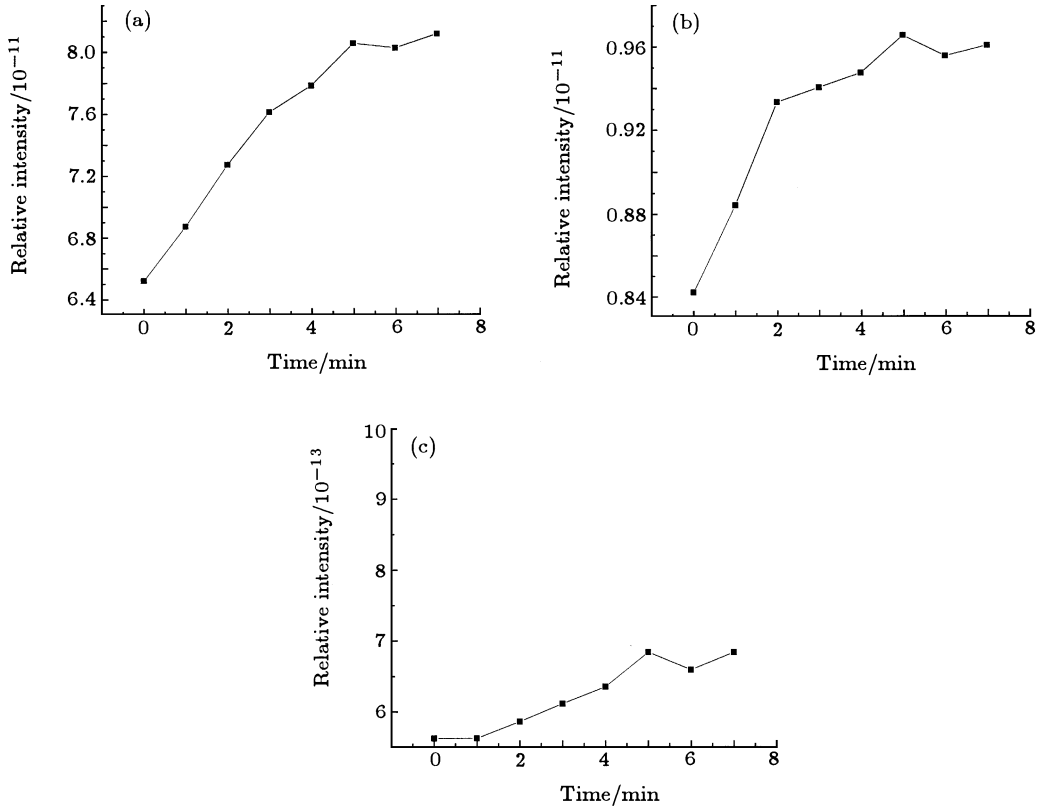


Fig.6. Intensity of several active species produced by APSBD: O_2 (a), O (b), and NO (c), plotted as a function of time.

Strangely, the most common product of DBD, ozone, could not be found in the mass spectrometer, but we could smell the characteristic odour of ozone even far from the discharge. The reason may be that ozone is unstable and when it enters the mass spectrometer, it decomposes very quickly,

$$O_3 + e \longrightarrow O_2 + O + e, \tag{1}$$

$$O + O_3 + M \longrightarrow 2O_2 + M. \tag{2}$$

The discharge generates ozone due to the reactions

$$O_2 + e \longrightarrow O + O + e, \tag{3}$$

$$O_2 + e \longrightarrow O + O^+ + 2e, \tag{4}$$

$$O + O_2 + M \longrightarrow O_3 + M, \tag{5}$$

where M can be N_2, O_2 or O, and excitation and dissociation of nitrogen molecules lead to a number of additional reaction paths such as

$$N + O_2 \longrightarrow NO + O, \tag{6}$$

$$N + NO \longrightarrow N_2 + O. \tag{7}$$

These reactions can produce additional oxygen atoms for ozone generation.

Ozone is a kind of strong oxidant, it can kill bacteria in different forms: it can decompose enzyme that is desirable in synthesizing glucose in bacteria; react with bacteria and virus directly and destroy cell wall, DNA and RNA of them; permeate the pericellular membrane and invade into the cell, destroy lipoprotein and lipopolysaccharide. The disinfectant process is from cell wall, pericellular membrane to cytoplasm and chromatin body, so the killing is very exhaustive. Ozone will degrade into molecular oxygen and monatomic oxygen, and the latter is another oxidant and some of the monatomic oxygen will combine into molecular oxygen, so they leave no harmful by-products at the end. Ozone, as a kind of gas, can pervade the whole space and leaves no dead angle.

Of course APSBD in air may generate other reactive oxygen species, such as O_2^-[14]

$$O_2 + e^- \longrightarrow O_2^-. \tag{8}$$

O_2^- is a highly toxic material and can destroy important high polymer and membrane of anaerobic bacteria's cell, but superoxide dismutase in the aerobic bacteria will turn it into H_2O_2, which then degrades into H_2O, so it does little harm to persons.

Since the ozone and monatomic oxygen are the

main antimicrobial products of APSBD, we can infer that this kind of discharge can be useful not only in killing *E. coli*, but also in killing a variety of microorganisms.[6] The wide application of APSBD is very promising.

4. Conclusions

The experiment on killing *E. coli* proves that the APSBD is a very simple, effective and innocuous tool for sterilization. The temperature is low and the process can be carried out in any conditions with only a portable high-voltage supply. There is almost no bacterial colony in the sample after being treated for 2 min. Ozone and monatomic oxide are considered to be the major antimicrobial active species and these species rapidly degrade into harmless products, leaving no harmful by-products.

Works are in progress to apply APSBD to sterilization such as decontamination of letters and generation of ozone water, and to further clarify the mechanism of bacterial destruction.

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