Disinfection of Escherichia coli in ice by surface dielectric barrier discharge plasma

Cite as: Appl. Phys. Lett. 119, 090601 (2021); doi: 10.1063/5.0064020
Submitted: 19 July 2021 · Accepted: 19 August 2021 ·
Published Online: 30 August 2021

Yuntao Guo,1 Peipei Liu,2 Liyang Zhang,1 Siqi Peng,1 Xinxin Wang,1 Haiyun Luo,1,a) and Guizhen Wu2,a)

AFFILIATIONS
1Department of Electrical Engineering, Tsinghua University, Beijing 100084, China
2NHC Key Laboratory of Biosafety, National Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China

Abstract

A variety of pathogens can cause people to suffer from serious diseases, and the transmission of COVID-19 through the cold chain has once again attracted people’s attention to cold chain disinfection. Unfortunately, there is no mature cold chain disinfection technique yet. In this study, a low-temperature plasma disinfection technique for a cold chain is proposed. The disinfection effect of plasma generated by surface dielectric barrier discharge on Escherichia coli in ice at cryogenic temperature is studied, and the possible disinfection mechanism is discussed. It is found that the O₃ mode and the NOₓ mode also exist in the surface dielectric barrier discharge at cryogenic temperature, just as at room temperature. The disinfection effect of both modes is weak in 5 min plasma treatment, but in 60 min post-treatment, the NOₓ mode shows a stronger disinfection effect, with 4.45 log reduction. It is speculated that gaseous H₂O₂ and NOₓ can be adsorbed on the ice surface and then converted into peroxynitrite, which is a powerful bactericidal species. In conclusion, a low-temperature plasma is a promising technique for cold chain disinfection, which is of great significance for ensuring people’s health.

Published under an exclusive license by AIP Publishing. https://doi.org/10.1063/5.0064020

ABSTRACT

A variety of pathogens can cause people to suffer from serious diseases, and the transmission of COVID-19 through the cold chain has once again attracted people’s attention to cold chain disinfection. Unfortunately, there is no mature cold chain disinfection technique yet. In this study, a low-temperature plasma disinfection technique for a cold chain is proposed. The disinfection effect of plasma generated by surface dielectric barrier discharge on Escherichia coli in ice at cryogenic temperature is studied, and the possible disinfection mechanism is discussed. It is found that the O₃ mode and the NOₓ mode also exist in the surface dielectric barrier discharge at cryogenic temperature, just as at room temperature. The disinfection effect of both modes is weak in 5 min plasma treatment, but in 60 min post-treatment, the NOₓ mode shows a stronger disinfection effect, with 4.45 log reduction. It is speculated that gaseous H₂O₂ and NOₓ can be adsorbed on the ice surface and then converted into peroxynitrite, which is a powerful bactericidal species. In conclusion, a low-temperature plasma is a promising technique for cold chain disinfection, which is of great significance for ensuring people’s health.

Published under an exclusive license by AIP Publishing. https://doi.org/10.1063/5.0064020

Escherichia coli, Staphylococcus aureus, human norovirus, and other foodborne pathogens can cause people to suffer from serious diseases. A cold chain plays an important role in food transportation and preservation, but concerns about food safety are often ignored. In the recent outbreak of COVID-19, Liu et al. reported that the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was directly isolated from the package surface of frozen cod, which was considered the source of infection in dock workers. Pang et al. speculated that the COVID-19 resurgence in Beijing was likely to be initiated by an environment-to-human transmission originated from contaminated imported food via cold-chain logistics. All the above cases are warning us that the contaminated foods in the cold chain could serve as a long-range carrier of pathogens, presenting a potential risk of its transmission across the regions and countries via cold chain industries. There is an urgent demand for disinfection in the cold chain to ensure the safety of the foods and the people involved.

Conventional thermal treatments can inactivate foodborne pathogens, but it obviously cannot be used for cold chain disinfection. In recent years, some non-thermal disinfection techniques have been developed, such as high-pressure treatment (HPP), ultraviolet (UV), ultrasonic, pulsed electric field (PEF), irradiation, and non-thermal plasma. However, there are few reports on disinfection techniques directly used in the cold chain. Baker et al. reported a per-oxygen based disinfectant in a 10% propylene glycol solution cloud inactivate epidemic diarrhea virus on aluminum surfaces at −10 °C. Zhang proposed the concept of using liquid CO₂ as a green and safe low-temperature disinfectant in the cold chain, but the disinfection effect was not verified by experiments.

Low temperature plasma, or named non-thermal plasma, is a novel disinfection technique with high efficiency and small side effects. Its disinfection effect has been widely studied, which can inactivate various foodborne pathogens such as bacteria, fungi, virus, and even SARS-CoV-2. Zhao et al. recently reported that the low-temperature plasma inactivated pseudoviruses with the SARS-CoV-2 S protein on the surface in a cold chain environment, but the study on the inactivation of pathogens in ice by the low-temperature plasma has not been reported. Due to the fact that the discharge process is almost independent of the ambient temperature, it is possible to generate a plasma by discharging at cryogenic temperature, which makes this technique has unique
advantages in cold chain disinfection. In this paper, the disinfection effect of the low temperature plasma on *Escherichia coli* (*E. coli*, ATCC 8099) in ice is studied, and the possible disinfection mechanism is discussed.

The experimental setup is shown in Fig. 1. Surface dielectric barrier discharge (SDBD) was used to generate the low temperature plasma in this study, which was similar to that of other researchers with slight changes.\(^{20,21}\) Briefly, a round aluminum plate (40 mm diameter), a stainless-steel mesh (40 mm diameter, 60 mesh/cm\(^2\)), and 1 mm ceramic plate were used as high voltage electrodes, ground electrodes, and dielectric barriers, respectively. A power source was used to supply a sinusoidal high voltage with the frequency of 1 kHz and the voltage amplitude in the range of 0–15 kV. The voltage and current were measured with a high voltage probe (Tektronix, P6015A) and a current probe (Tektronix, TCP312A), respectively. Another probe (Tektronix, TPP0200) was used to measure the voltage across a capacitor (65 nF) connected in series to the ground electrode. All waveforms were recorded using an oscilloscope (Tektronix, MDO3034). The power consumption was obtained by the Lissajous method\(^{24,25}\) and were recorded using an oscilloscope (Tektronix, MDO3034). The power consumption was obtained by the Lissajous method\(^{24,25}\) and then the power density was calculated by dividing the power consumption by the electrode area.

The power densities of SDBD at room temperature (30 °C) and cryogenic temperature (−20 °C) were measured, and the results are shown in Fig. 2(a). They are almost the same at the same voltage, and the difference is less than 7%. Taking 8 kV as an example, the shape and area of Lissajous figures [Fig. 2(d)] measured under these two temperatures are similar. The calculated power densities are 0.094 and 0.092 W/cm\(^2\), corresponding to −20 and 30 °C, respectively. When the voltage amplitude is 15 kV, the power densities are 0.327 and 0.319 W/cm\(^2\), respectively. As pointed out in the literature,\(^{24,25}\) the discharge area changes continuously during the SDBD process, resulting in the almond-shaped Lissajous figures.

The voltage and current waveforms of 8 and 15 kV at −20 °C are shown in Figs. 2(b) and 2(c). The current is composed of a series of waveforms, it is speculated that these two modes may also exist in the almond-shaped Lissajous figures. These two typical modes in the almond-shaped Lissajous figures. There are two typical modes of SDBD at room temperature, namely, the O\(_3\) mode and the NO\(_x\) mode, which has been found by other researchers.\(^{27,28}\) Shimizu et al.\(^{28}\) showed that the mode transition was related to the power density, and the threshold was about 0.1 W/cm\(^2\). Based on the results that the power density is almost the same at the same voltage at both temperatures, it is speculated that these two modes may also exist in the process of SDBD in the cryogenic environment.

UV spectroscopy is performed as an *in situ* measurement of the gaseous composition generated by SDBD at −20 °C. The schematic is shown in Fig. 1. A Teflon gas cell with a cylindrical cavity (40 mm in diameter and 15 mm in depth) in the center is designed. There is a hole (6 mm in diameter) with quartz glass on both sides at 5 mm below the ground electrode. Two optical fibers are connected to a mercury lamp (UV light source) and a spectrometer (HR2000+, Ocean optics), respectively. 253.74 and 435.89 nm are selected as the characteristic wavelengths of ozone and nitrogen dioxide, respectively. (Absorption cross sections are estimated to be 1.15 × 10\(^{-12}\) and 5 × 10\(^{-23}\) m\(^2\), respectively.)\(^{29,30}\) The spectral intensity of these two wavelengths during the discharge process is recorded, and then the gas concentration can be calculated by Beer’s law.

The concentrations of O\(_3\) and NO\(_2\) at −20 °C are measured within the first 5 min of discharge when the voltage is 8 and 15 kV, and the results are shown in Figs. 3(c) and 2(d). The evolution process of gas composition and concentration under these two voltages is obviously different. Specifically, when the voltage is 8 kV, the concentration of ozone continues to rise to more than 11 000 ppm within 300 s of discharge, while the concentration of nitrogen dioxide is less than 200 ppm in the whole process. In contrast, when the voltage is 15 kV, the concentration of ozone increases to about 750 ppm in 30 s, then gradually decreases to 0 at 150 s, while the concentration of nitrogen dioxide increases continuously especially after the reduction of ozone, and the maximum concentration can reach 3000 ppm. These results directly prove that there are the O\(_3\) mode and the NO\(_2\) mode in SDBD at cryogenic temperature. When the voltage is 8 and 15 kV, the power density is about 0.09 and 0.33 W/cm\(^2\), respectively, which is consistent with the power density threshold of mode transition proposed by Shimizu et al.\(^{28}\) They believe that the ozone depletion at high power density is caused by the quenching reactions of nitrogen oxides produced by the reaction of vibrationally excited nitrogen molecules with O atoms.

SDBD is an effective approach for direct surface disinfection\(^{27}\) and for indirect disinfection by plasma activated water.\(^{41}\) However, its direct disinfection effect on microorganisms in ice at cryogenic temperature is not clear. Schematic diagram of the plasma disinfection process at cryogenic temperature is shown in Fig. 3(a). First, 1 ml of *E. coli* normal saline solution with a concentration of ~1\(^{10}\) CFU/mL was put into a culture dish and frozen in a freezer for more than 12 h to prepare ice containing *E. coli*. Then, the dish was transferred into the gas cell, and plasma treatment of different time/power was carried out at −20 °C. After plasma treatment, the ice was little melted. It took at least 2 min at 37 °C for the ice to melt completely (up to 60 min is to study the disinfection effect of post-treatment time). Finally, the samples were serially diluted and plated on lysogeny broth (LB) agar followed by incubation overnight at 37 °C. Colonies were counted and converted to logarithmic values for evaluating the disinfection effect. The control group is the same volume of bacteria suspension, which is also frozen and melted without plasma treatment. All tests were performed in triplicate to provide a statistically robust comparison.

The disinfection effect of the O\(_3\) mode and the NO\(_2\) mode at cryogenic temperature is studied, including two factors: one is the plasma treatment time (1–5 min), and the other is the post-treatment time (2–60 min). The results are shown in Figs. 3(b) and 3(c). In general, the log of surviving *E. coli* decreased with the treatment time. As shown in Fig. 3(b), the disinfection effect of the two modes is weak.
under different plasma treatment time. (The post-treatment time is fixed at 2 min.) The log value only decreases by 0.67 and 1.22, respectively, in 5 min when the voltage is 8 kV ($\text{O}_3$ mode) and 15 kV ($\text{NO}_x$ mode). However, there is a significant difference in the disinfection effect between the two modes with the increase in post-treatment time. [As shown in Fig. 3(c), the plasma treatment time is fixed at 5 min.] The reduction of log value in the $\text{O}_3$ mode only increases slightly in 60 min (from 0.67 to 0.78), while it increases significantly to 4.45 in the same time in the $\text{NO}_x$ mode, showing a good disinfection effect.

Next, the possible disinfection mechanism of these two modes at cryogenic temperature is discussed. Heat and UV are not considered to be the main factors in the process of plasma disinfection, so the active species in plasmas are mainly considered here. Dozens of reactive oxygen species (ROS) and reactive nitrogen species (RNS) can be produced in the SDBD process, but according to the research results of Liu et al., most of the active species are short-lived species, and their diffusion distance is less than 1 mm. In this study, ice is placed 15 mm downstream of the SDBD setup, so only long-lived species are considered. As shown in Figs. 2(e) and 2(f), the gaseous products of the two modes are obviously different, one is mainly $\text{O}_3$ and the other is $\text{NO}_x$. Considering the relative humidity of 50% in the freezer, there is also the possibility of $\text{H}_2\text{O}_2$ and $\text{HNO}_x$. Shimizu et al. and Pavlovich et al. believed that ozone had a good disinfection effect, but the disinfection objects in their study were agar surface or solution. Ozone could directly act on bacteria or diffuse into a solution in their on.
different in the two modes. In the O\textsubscript{3} mode, the concentrations of the substances in the solution are significantly reagents (Beijing, Shanghai, China), respectively. The results show that methods of \textit{H\textsubscript{2}O\textsubscript{2}}, \textit{N\textsubscript{O\textsubscript{2}}}, and \textit{HNO\textsubscript{2}} can produce peroxynitrite under acidic conditions, which explains that \textit{H\textsubscript{2}O\textsubscript{2}} play a key role in their experiments, which explains that the disinfection effect has no obvious change with the increase in post-treatment time in the O\textsubscript{3} mode. When it comes to the NO\textsubscript{x} mode, the long-lived gaseous products may include NO\textsubscript{x}, H\textsubscript{N}O\textsubscript{x}, and NO\textsubscript{3}. In the 5 min of plasma treatment, as in the case of the O\textsubscript{3} mode, the disinfection only occurs on the ice surface, so the disinfection effect is also weak. With the increase in post-treatment time, peroxynitrite can diffuse in the solution and inactivate the \textit{E. coli} after ice melting, so it shows a good disinfection effect. The schematic diagram of the above assumed disinfection mechanism is shown in Fig. 4(a).

In order to verify the above hypothesis, concentrations of \textit{H\textsubscript{2}O\textsubscript{2}}, \textit{N\textsubscript{O\textsubscript{2}}}, and \textit{N\textsubscript{O\textsubscript{3}}} in the solution after melting ice treated by SDBD were further measured, and the curve of concentration with post-treatment time was obtained, as shown in Fig. 4(b). The detection methods of \textit{H\textsubscript{2}O\textsubscript{2}}, \textit{N\textsubscript{O\textsubscript{2}}}, and \textit{N\textsubscript{O\textsubscript{3}}} were described in Ref. 38 using Amplex Red Reagent (Genecopoeia, Rockville, USA) and Griess reagents (Beijing, Shanghai, China), respectively. The results show that the concentrations of the substances in the solution are significantly different in the two modes. In the O\textsubscript{3} mode, the concentrations of \textit{H\textsubscript{2}O\textsubscript{2}} and \textit{N\textsubscript{O\textsubscript{2}}} are about 3.5 and 1.5 \textmu{}M, respectively, and almost keep constant within 60 min post-treatment time. The concentration of \textit{NO\textsubscript{3}} slightly increases from 25 to 30 \textmu{}M. In the NO\textsubscript{x} mode, concentrations of \textit{H\textsubscript{2}O\textsubscript{2}} decrease from 9.5 to 8.5 \textmu{}M, the concentration of \textit{NO\textsubscript{2}} decreases from 13.5 to 10 \textmu{}M, and the concentration of \textit{NO\textsubscript{3}} slowly increases from 42 to 46 \textmu{}M. According to the third-order rate constant \(k = 1.1 \times 10^7 \text{ M}^{-1} \text{ s}^{-1}\) for the reaction \textit{NO\textsubscript{2}}\textsuperscript{−} + \textit{H\textsubscript{2}O\textsubscript{2}} + \textit{H}^{+} \rightarrow \text{ONOOH} + \textit{H\textsubscript{2}O},\textsuperscript{34} 254 \text{nm} peroxynitrite can be accumulated in 60 min post-treatment time in the NO\textsubscript{x} mode but only 10 \text{nm} in the O\textsubscript{3} mode (pH = 3.3). The 25-fold difference of peroxynitrite may be the main reason for the significant difference in the disinfection effect between these two modes.

In conclusion, a low temperature plasma disinfection technique for inactivating \textit{E. coli} in ice at cryogenic temperature was developed. The discharge characteristics of SDBD at cryogenic temperature (\(\sim 20\, ^\circ\text{C}\)) are almost the same as those at room temperature, and the power density at the same voltage is similar. Therefore, the mode transition of SDBD determined by power density is also found at \(\sim 20\, ^\circ\text{C}\), and it is the O\textsubscript{3} mode at 0.09 \text{W/cm}^{2} and the NO\textsubscript{x} mode at 0.33 \text{W/cm}^{2}. The disinfection effect of these two modes with different plasma treatment time and post-treatment time is investigated. It is found that the disinfection effect is not obvious within 5 min of plasma treatment in both modes, but it increases significantly with the post-treatment time in the NO\textsubscript{x} mode with 4.45 log reduction. It is speculated that \textit{H\textsubscript{2}O\textsubscript{2}} and \textit{NO\textsubscript{2}} can be adsorbed on the ice surface in the NO\textsubscript{x} mode and then converted into a powerful disinfection species, peroxynitrite, showing a good disinfection effect. The concentrations of \textit{H\textsubscript{2}O\textsubscript{2}} and \textit{NO\textsubscript{2}} in the solution are detected to verify the above hypothesis. Low-temperature plasma is a promising technique for cold chain disinfection, which is of great significance for the protection of people’s health and safety. The key disinfection factors and the disinfection mechanism in the cryogenic environment need to be further studied. In addition, a large-scale plasma disinfection device for practical applications needs to be further developed.

This work was supported by the National Key Research and Development Program of China (No. 2020YFC0862100) and the Tsinghua University Spring Breeze Fund (No. 2020Z99CFG007).

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

REFERENCES

\textsuperscript{1} B. Kim, H. Yun, S. Jung, Y. Jung, H. Jung, W. Choe, and C. Jo, Food Microbiol. 28, 9–13 (2011).
\textsuperscript{2} J. Kodarai, T. C. Smith, and D. Thapaliya, BioMed Res. Int. 2014, 827965.
\textsuperscript{3} H. A. Aboubakr, F. Sampedro Parra, J. Collins, P. Bruggeman, and S. M. Goyal, Food Microbiol. 85, 103307 (2020).
\textsuperscript{4} X. Liao, D. Liu, Q. Xiang, J. Ahn, S. Chen, X. Ye, and T. Ding, Food Control 75, 83–91 (2017).
\textsuperscript{5} D. D. Jayasena, H. J. Kim, H. I. Yong, S. Park, K. Kim, W. Choe, and C. Jo, Food Microbiol. 46, 51–57 (2015).
\textsuperscript{6} X. R. Zhang, Int. J. Energy Res. 45, 6483–6488 (2021).
\textsuperscript{7} P. Liu, M. Yang, X. Zhao, Y. Guo, L. Wang, J. Zhang, W. Lei, W. Han, F. Jiang, W. J. Liu, G. F. Gao, and G. Wu, Biosaf. Health 2, 199–201 (2020).
\textsuperscript{8} X. Pang, L. Ren, S. Wu, W. Ma, J. Yang, L. Di, J. Li, Y. Xiao, L. Kang, S. Du, J. Du, J. Wang, G. Li, S. Zhai, L. Chen, W. Zhou, S. Lai, L. Gao, Y. Pan, Q. Wang, M. Li, J. Wang, Y. Huang, and J. Wang, Natl. Sci. Rev. 7, 1861–1864 (2020).
