23P023

Inactivation Characteristics of Bacillus Thuringiensis Spore in Liquid Using Atmospheric Torch Plasma Generated by Low-frequency Discharge

大気圧トーチプラズマによる液中BT菌芽胞の不活化特性

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The atmospheric plasma torch produced by barrier discharge using air as working gas is adopted to sterilize microorganisms in liquid. The inactivation characteristics of bacillus spore are investigated comparing heat sterilization method, and sterilization factors are active species with short-lifetime produced in water. The population of *Bacillus Thuringiensis* spore in PBS solution decreases 10^{-3} times within 20 min, and the obtained result is equivalent to the heat sterilization of 95°C.

1. Introduction

Disinfection of microorganism in liquid is important procedure in medicine and food Industry. The unique method that has been adopted to inactivate the microorganism in liquid is the heat pulse method, which applies heat to liquid for short period of several minutes. The temperature of water increase to 130 °C, and microorganism including bacillus spores are inactivated. However, liquid to be sterilized tend to suffer modification in taste and aroma, in spite of short period of heat application.

Recently, atmospheric torch plasma using He gas has been adopted to inactivate microorganism in water[1,2]. This method serves the non-heat sterilization of liquid. Since the cost of He gas used in the method is significantly high, the He torch plasma sterilization method is difficult to be utilized practically. In this study, the atmospheric plasma torch using air is adopted to sterilize liquid. The inactivation characteristics of bacillus spore are clarified, and sterilization factors are attempted to be specified.

2. Experimental procedure

The plasma torch adopted in this experiment is categorized into the surface discharge electrodes. The tungsten wire as a grounded electrode is wound around an alumina tube of the length of 100 mm and the inner diameter of 3 mm, as illustrated in Fig. 1. Also, another tungsten wire is set inner surface of the alumina tube as a cathode. When the high voltage with the high frequency of 10 kHz is applied to the wire cathode electrode inside the alumina tube, the discharge occurs around the surface of the wire electrode. The air or pure oxygen gas is used as material gas of plasma. As the gas flows through the alumina tube, the ions and radicals are ejected from the opening edge of the alumina tube. The distance between the opening edge of the torch tube and the liquid surface is 15mm, in order to prevent evaporation of the liquid by the gas flow. The applied voltage and the gas flow rate are kept constant, 6 kV and 1 slm, respectively.

The bacillus spore utilized as sample microorganism in the PBS is the *Bacillus Thuringiensis* that is tolerant against heat and UV light. The vegetative state of the *Bacillus Thuringiensis* is also served, and its inactivation characteristics are compared with those of the spore. The population of the spore is order of 10^6, which is dispersed in pure water. The torch plasma irradiates to the surface of the water in the small petri dish. The amount and depth of the water are 20ml and 3mm, respectively.

3. Results and discussion

3.1 *Production of active species*

Figure 2 shows the typical open-shutter photograph of the air torch plasma. The light emission of the plasma torch is observed with the length of approximately 5 mm, which is mainly originated from the excited nitrogen. Figure 3 illustrates the light emission spectra measured using a multi-channel spectrometer. The nitrogen second

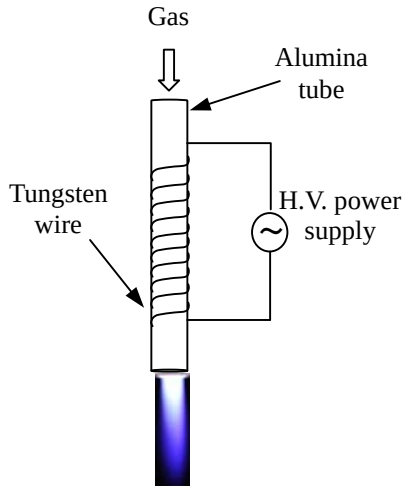
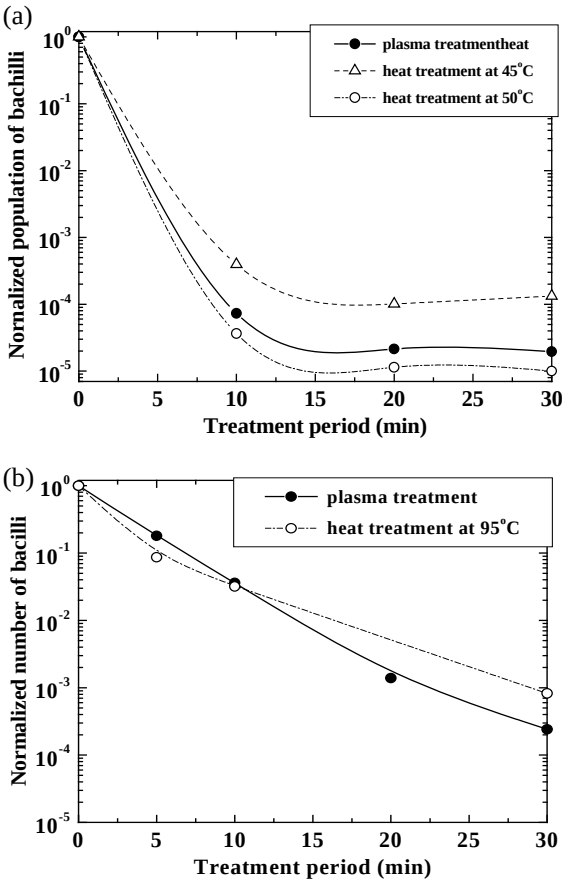


Fig.1. Schematic of atmospheric pressure torch plasma apparatus.



Figs.2. Survival curves of the vegetative (a) and spore (b) of *Bacillus Thuringiensis* using plasma and heat treatment.

positive band is significant in the spectra. Therefore, the nitrogen meta-stable particles would affect the bacillus in the solution. Also, a chemical indicator for oxygen radical detection indicates the generation of active oxygen species at the down -stream of the torch plasma.

In general, when the atmospheric air or Ar plasma irradiates to the water surface, the hydrogen peroxide (H_2O_2) is generated in the water due to the dissociation of H_2O by high-speed neutral particles. In the case of oxygen plasma, velocity of particles ejected from the torch would be small, and H_2O_2 has not been detected throughout the plasma irradiation for 20 min using H_2O_2 test strips (Merquant). Also, the dissolved nitric oxide is not obtained in the water after the discharge for 20 min.

The Ultra violet (UV) light emission from the plasma torch is measured utilizing the UV detection label, which is located at the bottom of the liquid vessel. The UV label is irradiated by the plasma torch, and indicates that the UV light emission intensity from the plasma torch is significantly smaller than that of the UV light tube for microorganism inactivation. The temperature of both of the water and the gas on the surface of the water is below 40 ^{o}C, which is 15 ^{o}C higher than the room temperature. This temperature is acceptable for the vegetative state of *Bacillus Thuringiensis*. Above results imply that the sterilization factor is not active species of H_2O_2, N_2O^- and O_3 and UV light, but unidentified active species with short lifetime, such as OH radical.

3.2 *Inactivation of bacillus in liquid*

Figures 2(a) and (b) illustrate survival curves of vegetate state and spore of bacilli with irradiation of torch plasma, respectively. Dashed lines indicate survival curves of heat inactivation. The effects of the plasma irradiation is equivalent to the heat inactivation with the temperature of 50^{o}C and 95^{o}C in the case of vegetate state and spore of bacilli, respectively. When the oxygen flow rates are 1 l/min and 3 l/min, the inactivation effects of the plasma irradiation are equivalent to the heat inactivation with the temperature of 95^{o}C and 85^{o}C, respectively. Above all tendencies of the survival curves with the plasma treatments are similar to those with the heat treatment. This fact implies that the inactivation mechanism by the plasma irradiation would be same as the heat treatment that is modification and decomposition of proteins containing inside the cell wall.

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