Inactivating *C.sphaerospermum* and its mechanism in atmospheric pressure plasmas with water mist

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Sterilization technology is crucial in many industries, and there are currently several methods used to achieve it. However, selecting the appropriate sterilization method is essential and depends on various factors such as toxicity, temperature, and other issues related to the object to be treated. Atmospheric pressure plasma sterilization is a promising general-purpose sterilization method that can overcome these issues and sterilize materials with low toxicity and low-temperature conditions.

In this study, we aimed to elucidate the mechanism of inactivation of *C.sphaeospermum* using atmospheric pressure plasmas with water mist. The inactivation system used in this study consisted of a mist generator and a discharge device. The mist generator used an ultrasonic transducer to convert liquid water in a tank into mist, and the discharge device consisted of two stages of two rows of copper wire covered with ceramic. It was connected to high voltage in an upper part and to the ground in a lower part to generate plasmas by applying an AC voltage of 13 kVpp. The water mist was carried by dry air at a flow rate of 1.0 L/min and blown through the plasma onto the object (*C.sphaeospermum*) to be treated. We examined the system's inactivation ability by counting the number of surviving cells after treatment on a plate medium coated with *C.sphaerospermum*. Additionally, the treatment was conducted with and without water mist in the gas, and the results were compared.

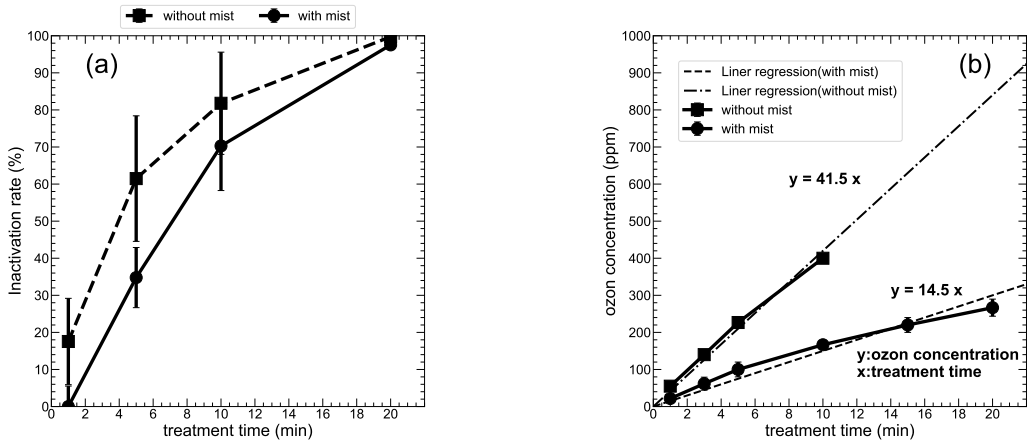

Fig. 1: (a) inactivation rates of *C.sphaerospermum* as a function of treatment time in the treatments without and with water mist, and (b) ozone concentration as a function of the time.

Figure 1 (a) depicts the relationship between the treatment time and the inactivation rate of *C.sphaerospermum*. It is indicated that with an increase in treatment time, the inactivation rate of *C.sphaerospermum* also increases. The graph also indicates that the inactivation rate of *C. sphaerospermum* reaches 99% within 20 minutes, regardless of whether mist is present or not. Figure 1 (b) shows ozone concentration versus treatment time. The graph shows that ozone concentration increases as the treatment time increases. However, the detector tube used to measure ozone concentration had a limit of 400 ppm, so it was not possible to measure the ozone concentration in the treatment without water mist after 10 minutes. Therefore, the ozone concentration after 20 minutes was estimated from the measurement result using a regression line. The graph reveals that the mist inclusion in the gas reduces the increase in ozone concentration by approximately one-third.

The OH radical, which has a strong oxidizing ability than ozone, is one of excited species produced by the plasma with water mist. The radical was detected by a chemical probe method. In this study, gels made from the fluorescent reagent NaTA solution were treated with the inactivation system and fluorescence was observed. Figure 2 shows the fluorescence intensity of treated and untreated NaTA gels. It is indicated that the fluorescence intensity of the gel was shown to be enhanced by treatment with mist-containing gases.

The results showed that *C. sphaerospermum* could be inactivated 99% by the treatment with an atmospheric pressure plasma for 20 min. In the treatment with the atmospheric pressure plasma without water mist, inactivation is considered to be caused by exposure to ozone generated from oxygen molecules. However, the plasma with water mist had the same inactivation rate while the ozone concentration was less than that of the plasma without water mist. We measured the OH radical and observed that the amount of the OH radical produced increased in the plasma with water mist. Therefore, the OH radical in addition to ozone is responsible for inactivation of *C.sphaerospermum* by the atmospheric pressure plasmas with water mist.

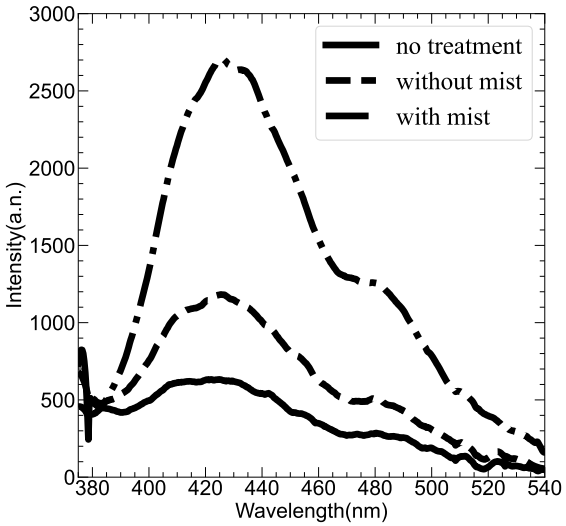

Fig. 2: Intensities of fluorescence from NaTA-containing gels in wavelength between 380 and 540 nm.