Spectroscopic characteristics in non-equilibrium atmospheric pressure plasma for inactivation of micro-organism

Takayuki Ohta1, Sachiko Iseki2, Masafumi Ito3
Hiroyuki Kano4, Yasuhiro Higashijima5, Masaru Hori2

1 Faculty of Systems Engineering, Wakayama University, 930, Sakaedani, Wakayama 640-8510, Japan
2 Department of Electrical Engineering and Computer Science, Nagoya University, Furo-cho, chikusa-ku, Nagoya 464-8603, Japan
3 Faculty of Science and Technology, Meijo University,1-501, Shiogamaguchi, Tenpaku-ku, Nagoya 468-8502, Japan
4 NU EcoEngineering CO. LTD., 1237-87, Kurozasa, Miyoshi-cho, Nishikamo-gun, Aichi 470-0201, Japan
5 NU system CO. LTD., 2271-129 Shimoshidami, Moriyama-ku, Nagoya 463-0003, Japan

Abstract: Spectroscopic characteristics in non-equilibrium atmospheric pressure plasma were investigated in order to clarify the inactivation of Penicillium digitatum. The emissions of N2 and NO were observed in ultraviolet region. It is found that the UV light produced by the plasma is not a dominant effect on the plasma inactivation in atmospheric pressure. The behavior of emission intensity of O atom corresponded to the inactivation rate, which indicates that O-related radicals are important species for the inactivation of Penicillium digitatum.

Keywords: non-equilibrium atmospheric pressure plasma, inactivation, Penicillium digitatum

1 Introduction
In agricultural field, methyl bromide, which is the one of effectively pesticides, is sprayed to the crops for protecting from various insects and virus. Methyl bromide is an ozone depletion material and had been forbidden to be used since 2005, based on "Montreal Protocol on Substances that Deplete the Ozone Layer". However, the useful substitute-technology has not been developed yet. Conventional inactivation techniques are autoclaves, ovens, and chemicals such as ethylene oxide, hydrogen peroxide, etc. However, their chemicals cause some harmful problems for the human body and environment due to its toxicity. In the cases of autoclaves and ovens, the heat damages to crops become a problem. The establishment of substitute-technology has been required.

Recently, inactivation of micro-organisms using plasma has attracted much attention, especially for medical instruments. In low-pressure plasma, the cost becomes high for the agriculture due to vacuum systems. The atmospheric pressure plasma has been also used for inactivation of microorganisms. The inactivation mechanism of bacteria and virus by using plasmas was reported to be the DNA damage by ultraviolet (UV) light and the oxidation of a cell membrane by O3, O radical and OH radical [1][2]. It is important to investigate the mechanism of the inactivation in order to treat effectively.

We have used spores of Penicillium digitatum as a sample which causes green mold disease of the crops such as citrus fruits. P. digitatum was successfully inactivated by high electron density non-equilibrium atmospheric pressure plasma which was developed by our research group [3]. The inactivation was enhanced by O2 gas addition. This result indicated that O-related radicals would be one of important species for the inactivation of P. digitatum [3]. In this study, the diagnostics of non-equilibrium atmospheric pressure plasma was performed in order to clarify the inactivation mechanism of P. digitatum. Emission spectra from non-equilibrium atmospheric pressure plasma was measured. The emission intensities of O and Ar were measured in various Ar/O2 mixture gas ratios. Moreover, the inactivation effect of UV light from non-equilibrium atmospheric pressure plasma was investigated.

2 Experimental
P. digitatum was inactivated using non-equilibrium atmospheric pressure plasma. Figure 1 shows the schematic diagram of the plasma inactivation. The size of this plasma source is 20mm in diameter and 50mm in height. Ar gas of 3L/min was flown through the gap between the
electrodes. The plasma in this study was non-thermal plasma due to high speed flow rate. The electron density of this plasma source was reported to be $10^{15}$ cm$^{-3}$ [4]. Sample spores were diluted by inactivated water. The sample of diluted spores of 1ml was dripped on the cover glass and placed under the electrodes of plasma source. After plasma exposure, the samples were collected from the cover glass and diluted in inactivated water. The diluted samples were cultured on a culture medium in an incubator at 25 °C for 72 hours. For evaluating the inactivation by plasma exposure, the colony count method was used. In order to avoid the inactivation by plasma heating, the sample was set on more than 10mm apart from the electrodes. The temperature of the sample at 10mm was kept below 60 °C.

3 Spectra of Non-equilibrium atmospheric pressure plasma

Spectroscopic characteristics of non-equilibrium atmospheric pressure plasma were measured by optical emission spectroscopy (OES). Figure 2 shows the schematic diagram of OES. Optical system was composed of a fiber-coupled lens, an optical fiber and a multi-channel spectrometer. The spatial resolution of the measurement was approximately 1mm. The measurement was performed at temperatures of 11-30 °C, and relative humidities of 20-45%.

Emission spectra of the plasma were shown in Fig. 3 to Fig. 5. Figure 3 shows emission spectra from 250nm to 280nm. The emissions of ($N_2$, 5$\text{th}$ positive system; D$^3\Sigma^+$ $\rightarrow$ B$^1\Pi$) and NO ($\gamma$ system; A$^3\Sigma^+$ $\rightarrow$ X$^2\Pi$) were observed, which indicates that UV light was emitted from the non-equilibrium atmospheric pressure plasma. Emission spectra from 310 to 315nm were shown in Fig. 4. Emission spectra of OH radical at 306.3, 306.7, 307.8, 308.9 nm (OH 3064Å SYSTEM; A$^3\Sigma^+$ $\rightarrow$ X$^2\Pi$) were observed. OH radical was generated from ambient air by the non-equilibrium atmospheric pressure plasma. Emission spectra from 740 to 790 nm were shown in Fig.

$$O_2 + e \rightarrow 2O + e, \quad (1)$$
$$O + O_2 + M \rightarrow O_3 + M. \quad (2)$$

where, e is the electron, M is the gas molecule.

As shown in Fig. 3 to Fig. 5, the emissions of N$_2$, NO, OH, Ar and O were observed. In this study, non-equilibrium atmospheric pressure plasma was mainly generated by Ar in the atmospheric pressure. These results indicate that N$_2$, O$_2$, H$_2$O (vapor) in the air were dissociated by non-equilibrium atmospheric pressure Ar plasma. Thus UV light, and species such as O and OH radical can be a possible inactivation factor.
te for the inactivation effect by UV light, the quartz plate was set between the plasma and sample. This plate was used for shutting radicals and O₃ away from the samples. The sample spores were set at a distance L from the electrodes to sample of 20 mm. Compared with the UV light inactivation, the plasma inactivation without quartz plate, which included radicals and O₃, was also performed.

Figure 7 shows the result of UV light inactivation. The D value (Decimal reduction time) of UV light inactivation using the non-equilibrium atmospheric pressure plasma was 26.2 min. On the other hand, the D value of plasma inactivation which included radicals and O₃ was 5.8 min. This result indicated that the contribution of UV emission to the inactivation was not dominant.

5 Effects of O₂ gas addition

O₃ and O radical are produced from the plasma as mentioned in sec. 3. However, the contribution of O₃ to the inactivation was concluded to be small from our previous report [3]. In order to investigate the effect of O radical for the inactivation, O₂ gas was added to the Ar (3 L/min) gas. Figures 8 and 9 show the emission intensities of Ar (750 nm) and O (777 nm) as a function of the distance from the electrodes, respectively.
nsity of Ar decreased with an increase in the O₂ gas flow rate while that of O increased with an increase in the O₂ gas flow rate from 0 to 100 sccm and then decreased at an O₂ gas flow rate of 1000 sccm.

In order to investigate the inactivation effect of O₂ gas addition, P. digitatum was exposed by the plasma employing Ar/O₂ mixture gas. The O₂ gas flow rate was varied from 0 to 100 sccm. The sample was set at a distance L from electrode of 20 mm. Figure 10 shows the number of survivors as a function of plasma exposure time at various O₂ addition flow rates. D values at O₂ flow rates of 0, 10, 50, and 100 sccm were estimated to be 5.0, 2.1, 1.5, 1.6 min, respectively. The D value decreased with an increase in the O₂ gas flow rate from 0 to 50 sccm, and was saturated over 100 sccm. The behavior corresponded to that of O emission intensity. These results indicate that the production of O related radical is effective to the inactivation.

6 Conclusion

In this study, spectroscopic characteristics of non-equilibrium atmospheric pressure plasma were investigated. The emission spectra of N₂ and NO were observed in the UV region (250-280 nm). P. digitatum was exposed to UV light from the plasma, and it is found that the contribution of UV light to the inactivation were not dominant. Emission spectra of OH and O were also observed. OH and O radical were generated by the non-equilibrium atmospheric pressure plasma. The emission intensity of O increased with an increase in the O₂ gas flow from 0 to 100 sccm. On the other hand, emission intensity of Ar decreased with an increase in the O₂ gas flow rate. Moreover, D value decreased with an increase in the O₂ gas flow rate from 0 to 50 sccm. From these results, it was concluded that O related radicals would be one of important species for the inactivation of P. digitatum.

7 References