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Effect of Atmospheric Plasma Irradiation to Yeast Fungus and Colon Bacillus
酵母および大腸菌への大気圧プラズマ照射の影響

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Atmospheric plasma has taken an important part for practical applications for plasma sterilization. Plasma jet and surface discharge as typical atmospheric plasma source were applied to eliminate microorganisms in the study. No colonies around the point of exposing plasma jet and surface discharge were observed, and the area of it tends to depend on irradiation period and species of microorganism. It was supposed that some ambient molecules were converted into active species for sterilization agent by atmospheric plasma such as ozone, singlet oxygen molecule and nitrogen oxide. Furthermore, inhibition area of forming colony tended to become larger with irradiation of surface discharge in comparison with plasma jet.

1. Introduction

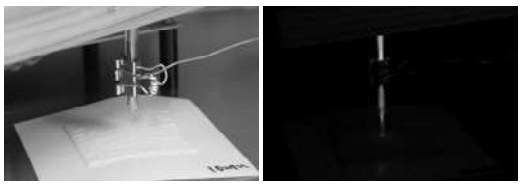
Active species generated in plasma have been become a major focus for sterilization as substitution of practical sterilization method using high pressure steam (autoclave sterilization) and ethylene oxide gas (EOG sterilization, C_2H_4O) [1]. Simultaneously, mechanism and novel application of plasma sterilization have been also studied actively in a decade [2][3].

Phenomena of plasma irradiation to biological objects, such as living body, fruits and vegetables, have become one of the major topics. Atmospheric plasma has taken an important part for practical applications because a target of plasma sterilization has difficulties to keep characteristics under low pressure. Plasma jet and surface discharge as typical atmospheric plasma source were applied to eliminate microorganisms and effect of atmospheric plasma irradiation is reported in this study.

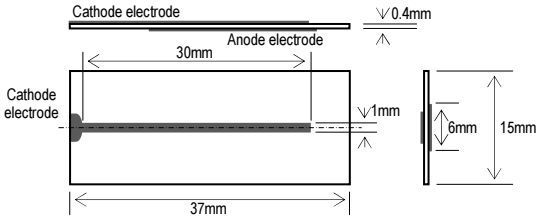
2. Experimental instruction

Atmospheric plasma equipments are applied to this study, and AC high voltage power unit (Logy electric Co., LHV-10AC) rated 10kV with 9kHz to 11kHz is prepared as stable source of power. Typical plasma jet equipment shown in Fig. 1 and surface discharge element shown in Fig. 2 were applied to this study. Plasma jet was generated with an electrode placed around φ5mm glass tube. Helium gas flow of each glass tubes was kept on 0.5L/min consistently. Surface discharge element made of ceramic plate (37mm long × 15mm wide × 0.4mm thick) placed between electrodes made of evaporated tungsten. Anode has 22mm long × 6mm wide with 100μm thick and cathode has 30mm long × 1mm wide with 100μm thick. Surface discharge element was arranged parallel in four lines. Irradiation period of atmospheric plasma is varied from 0min to 10min.

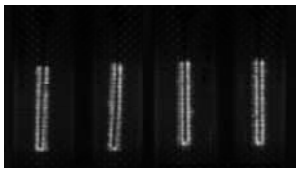
Two species of microorganisms were prepared for irradiation of atmospheric plasma. Yeast fungus (*Saccharomyces cerevisiae*, NBRC No.2347) and colon bacillus (*Escherichia coli*, NBRC No.3972) were inoculated into liquid culture medium and applied to plasma treatment as object of the study.



(a) Before ignition (b) After ignition
Fig. 1. Atmospheric helium plasma jet



(a) Details of surface discharge element



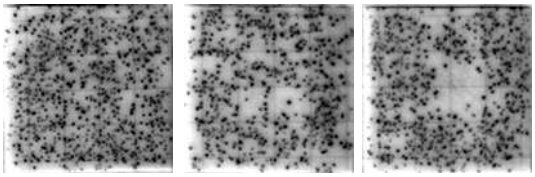
(b) Surface discharge element arranged parallel to each other
Fig. 2. Surface discharge element

Effect of atmospheric plasma irradiation was evaluated with commercially available cultural medium sheet named Sanita-kun (Chisso Corp.) for "coliform group" and "yeast & mold (Rapid Type)". Active microorganism grows and forms colony on the surface layer of cultural medium sheet, and the colony of microorganism colors with chromogenic reaction. Yeast fungus and colon bacillus in liquid culture medium, whose initial number was adjusted in 4×10^3 CFU/ml, were applied onto cultural sheet medium with 1ml sample solution, and then atmospheric plasma was irradiated near the centre of cultural sheet medium. After exposing atmospheric plasma to microorganisms, cultural sheet medium for "coliform group" and "yeast & mold (Rapid Type)" were incubated at 35°C for 24 hours and 25°C for 48 hours, respectively.

3. Experimental results

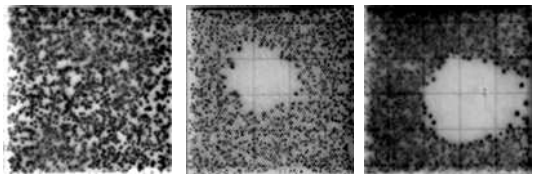
3.1 *Plasma jet*

Typical cultural medium sheets of yeast fungus and colon bacillus after exposing plasma jet are indicated in Fig. 3. It is found that ring of inhibition around the point of exposing plasma jet and the diameter of it tends to depend on irradiation period and species of microorganism. Yeast fungus tends to have tolerance against plasma exposure in comparison with colon bacillus because each microorganism has different structure of cell walls, and reactions of plasma to cell walls are supposed to depend on the species. Plasma jet with helium gas was exposed into air and it involved especially oxygen or nitrogen. Therefore, some ambient molecules convert into active species for sterilization agent such as ozone, singlet oxygen molecule and nitrogen oxide.



0min(cont.) 5min 10min

(a) Yeast fungus, *Saccharomyces cerevisiae*



0min(cont.) 5min 10min

(b) Colon bacillus, *Escherichia coli*

Fig. 3 Cultural medium sheets (Sanita-kun, Chisso Corp.)after plasma jet irradiation

Physiological saline was applied to cultural sheet medium instead of 1ml sample solution which was pure water. Inhibition ring of colon bacillus with physiological saline tended to be larger than applying pure water although yeast fungus is almost same irrespective of sample solution (Fig. 4). It is supposed that sterilization agents, such as ClO^-, HClO and $HClO_2$, were generated when plasma jet emitted to physiological saline.

3.2 *Surface discharge*

Surface discharge was irradiated to yeast fungus when surface discharge elements close to cultural medium sheet. It was clearly found that colonies did not form near surface discharge and inhibition area tended to increase with duration of irradiation of treatment. Inhibition area of forming colony became larger with irradiation of surface discharge compared to plasma jet. However, effective area of plasma sterilization should be larger for practical usage.

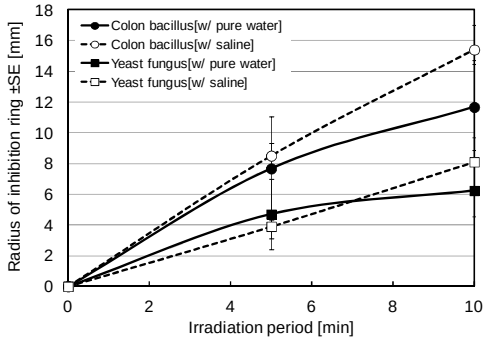
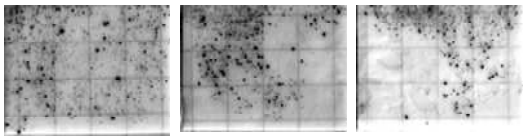


Fig. 4 Relationship between irradiation period of plasma jet and radius of inhibition ring.



0min(cont.) 2min 5min

Fig. 5 Cultural medium sheets after Surface discharge irradiation

Acknowledgments

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