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Plasma sterilization using glow discharge at atmospheric pressure

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Abstract

Recent development of atmospheric pressure glow discharge was compared with the performance of an apparatus used in the first APG experiment, in terms of sterilization of newly classified biological indicator: *Bacillus atrophaeus*, former *Bacillus subtilis var. niger* and *Geobacillus stearothermophilus.* Stabilization was attained by controlling the experimental conditions, at low frequency: 100 kHz and Radio Frequency: 13.56 MHz, water vapor/He dilution. Large volume of meta-stable atomic helium is responsible for the result that aids generation of hydroxyl radicals.

Keywords: Atmospheric pressure glow (APG); Plasma sterilization; *Bacillus atrophaeus*; *Geobacillus stearothermophilus*

1. Introduction

Low temperature plasma sterilization is attracting attentions as safe and short cycle cleaning technique as an alternative method for the chemical sterilization, using ethylene-oxide gas(EOG). Sterilization is inactivation of microorganisms, at factory of medical-care industry and hospital, as a part of prevention of infection and sterility assurance system. Widely accepted conventional schemes can be classified into two categories, high temperature process: pressurized steam treatment, autoclave, and dry-heat treatment and low temperature sterilization process: gas sterilization and radiation sterilization. Steam sterilization is suitable for sterilization of metallic and glass-made object. Heat treatment is not suitable for materials that have low resistance to heat. Distortions of plastic parts and deterioration of surgical knives are experienced.

Gas sterilization is a treatment using gaseous compound, ethylene oxide. This process allows low temperature disinfection of materials for medical treatment. Currently, this process is carrying the part of central pillar in low temperature sterilization process. Nevertheless, residual gaseous agent influences patients and medical operators toxically, and strong concern to human carcinogenicity is expressed since the early 1990s [1,2]. Thus, environmental emission is strongly restricted by PRTR law, Pollutant Release and Transfer Resistor law, 2000, in Japan.

Sterilization process using hydrogen-peroxide plasma is a scheme developed in the 1980s, performed by a batch process in vacuum chambers, using hydrogen-peroxide flush followed by back diffusion RF plasma. This scheme was approved by former Ministry of Health and Welfare in Japan in1994, and is coming into wide use as an alternative approach for the ethylene-oxide gas sterilization. In a rigorously scientific definition, major mechanism for sterilization is chemical sterilization by hydrogen peroxide vapor.

The present work aims to describe our plasma sterilization system using pulse modulated radio frequency, 27.12 MHz, under the atmospheric pressure. The idea of atmospheric pressure glow was first developed by S. Okazaki and her researchers group. First report was presented in 1987 in The International Symposium on Plasma Chemistry (ISPC-8) Tokyo conference. The atmospheric pressure glow (APG) plasma was first described by Kanazawa et al in 1987, 1988, 1989, Okazaki and Kogoma in 1989 [3–7]. The APG plasma method is applicable to majority part of plasma treatments at

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atmospheric pressures because of its operational low cost compared with the chamber-type, low-pressure plasma. With an attitude to find out new applications for this old knowledge, we compared the sterilizations characteristics of the original apparatus used in the early stage of the APG research. The original apparatus is conserved in good operational condition in Sophia University. Study on the antibacterial effect was carried out using RF glow discharge, at 100 kHz and 13.56 MHz; using two type of spore-forming bacteria: *Bacillus atrophaeus* and *Geobacillus stearothermophilus*. Although acceptable RF power is quite limited, for the original apparatus, still we can find out that higher frequency was capable to exhibited shorter cleaning cycle in the terms of the antibacterial effect. New system can deliver pulse modulated RF power at 27.12 MHz, up to 1 kW, to larger volume, realizing shorter sterilization time without thermal deterioration.

In these experiments, helium gas, working gas, has principal effect for the homogeneously excited plasma at high pressures, because of its low breakdown voltage. Helium atoms have high-energy meta-stable states. The energy transfer excitation is responsible for the expansion of the micro-discharges on the insulating plate. The excitation of mixed water molecules to generate free-radicals, such as hydroxyl radicals

$$\mathrm{He} + \mathrm{electron} \rightarrow \mathrm{He}^*(2^3\mathrm{S}, 2^1\mathrm{S}) + \mathrm{electron}$$

$$\mathrm{He}^* + \mathrm{H_2O} \rightarrow \mathrm{He} + \mathrm{HO} + \mathrm{H}$$

and atomic oxygen.

$$\mathrm{He} + \mathrm{electron} \rightarrow \mathrm{He}^*(2^3\mathrm{S}, 2^1\mathrm{S}) + \mathrm{electron}$$

$$\mathrm{He}^* + \mathrm{O_2} \rightarrow \mathrm{He} + \mathrm{O}^* + \mathrm{O}$$

These oxygen radicals react with the cell-wall of microorganism and destroy its double helix structure of DNA.

2. Experimental apparatus

In the original version, the electrical discharge was generated between plane metallic electrodes covered with optical flat glass, 50 mm in diameter. The gap between the electrodes was 5 mm. The entire vacuum system is constructed using glass tube and vessels. Fig. 1 shows the experimental setup. Plasma was excited at two frequency ranges: audio-frequency range 100 kHz, 80 W, and radio frequency range 13.56 MHz, 150–200 W. These two operational conditions were selected as typical examples for low damage, moderate treatment for soft materials such as cellulose sheet and short treatment for heat-resisting materials such as metallic tool; at 13.56 MHz. These conditions were selected as one of best parameters for each process considering the limited heat-resistance of glass vessel and electrodes. Our intention is not the empirical discovery of power scaling law.

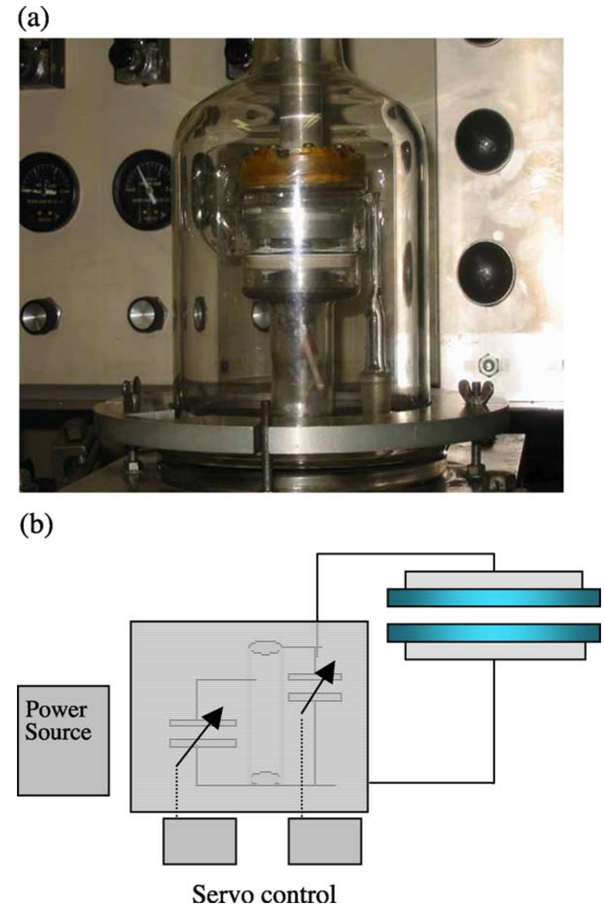

Fig. 1. Experimental setup for the APG plasma sterilization. (a) Electrodes, glass vessel and storage for distilled water for the vapor flow. (b) The circuit diagram and a schematic presentation for the structure of the electrodes (Original APG apparatus).

Temperature of the glass (insulator) covering the electrode was measured with Infra-Red Thermometer through a BaF_2 optical window. Saturated water vapor was supplied as source for reactive oxygen radicals.

New application for an experimental comparison, a pulse-modulated RF APG, at 27.12 MHz, was constructed (Fig. 2). Anti-bacterial effect was validated using biological indicators: spore-forming bacteria: *B. atrophaeus* ATCC9372 and *G. stearothermophilus* ATCC7953, and selected species of opportunistic pathogen: *Escherichia coli* ATCC8739, *Salmonella enteritidis*, *Staphylococcus aureus* ATCC6538, *Candida albicans* ATCC10231. The discharge volume between two dielectric barrier discharge electrodes has a dimension of 150 mm in length, 50 mm in width, and 3 mm gap The whole installation consists of linear actuator for programmable transportation of samples of microorganisms and a RF power generator, 670 W, at industrial frequency: 27.12 MHz. Electrodes are cooled by circulation of chilled water circulating inside the aluminum blocks. Metallic parts of upper and lower electrodes were covered with transparent fused quartz plate of size, 230 mm in length×115 mm in width×3 mm in thickness. Dielectric