



Sterilization effect of nitrogen oxide radicals generated by microwave plasma using air



Tomomasa Itarashiki ^{a, b, *}, Nobuya Hayashi ^a, Akira Yonesu ^c

^a Interdisciplinary Graduate School of Engineering Sciences, Kyushu University, 6-1 Kasuga-koen Kasuga-shi, Fukuoka 816-8580, Japan

^b Saraya Co. Ltd., 2-2-8 Yusato, Higashisumiyoshi-ku, Osaka 546-0013, Japan

^c Faculty of Engineering, Ryukyu University, 1 Senbaru Nishihara-cho Nakagami-gun, Okinawa 903-0213, Japan

ARTICLE INFO

Article history:

Received 1 November 2013

Received in revised form

5 June 2014

Accepted 16 June 2014

Available online 26 June 2014

Keywords:

Microwave plasma

Medical sterilization

Active oxygen species

RF plasma

ABSTRACT

Plasma sterilization has attracted attention as a safe and low cost sterilization method compared to conventional sterilization methods such as EOG (ethylene oxide gas) sterilization and high-pressure steam sterilization. In this research, a plasma is generated by irradiating air with microwaves. Sterilization is caused by O-radicals and NO-radicals. In order to carry out sterilization efficiently, optimal setting of equipment conditions is required. The pressure required for optimal conditions of the equipment, the microwave output, and the equipment used have been examined. A biological indicator (BI), *Geobacillus stearothermophilus* (ATCC7953), was used. It was found that sterilization was successful when the pressure in the vacuum chamber for microwave output of 280 W was 60 Pa.

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1. Introduction

Medical implements are typically washed and then sterilization is performed prior to their use. A high-pressure steam sterilization machine, an EOG (ethylene oxide gas) sterilizer, or a hydrogen peroxide gas sterilizer are typically employed. Heat-resistant and damp-proof medical implements are sterilized using the high-pressure steam sterilization machine. Other equipment that is not heat-resistant is sterilized with an EOG sterilizer or a hydrogen peroxide gas sterilizer. However, since a hydrogen peroxide gas sterilizer has a high operating cost, the EOG sterilizer is generally used as the first choice. While an EOG sterilizer is used at low temperatures, it could be carcinogenic [1]. Removal of residual toxic gases takes 8 h or more. Considering the capacity and cost of sterilization, EOG sterilization is still the method of choice. Moreover, in the case of the hydrogen peroxide gas sterilizer, although hydrogen peroxide is not carcinogenic, when used at high concentrations, there is danger of explosion and burns. Moreover, decomposition of the hydrogen peroxide after processing is also not complete.

It has been reported that residual EOG and hydroxyl peroxide could result in a negative effect. At medical facilities, sterilization

processing at low temperatures with no burden to the environment along with high safety and low cost is desired [2]. Although some reports concerning plasma sterilization using oxygen, argon, helium or their mixture have been published, there is no detailed study on the medical sterilization using atmospheric air plasma. Also, there are reports, which investigate inactivation of thermophiles using microwave plasmas. However, the study on reduction of thermophiles in a vacuum chamber has not yet been published [3–9]. In this study, since oxygen radicals generated in air plasma are used, good safety is achieved and there is no environmental burden. Therefore, sterilization processing at low temperatures is possible and can substitute for EOG sterilization, for sterilization of non-heat-resistant material, and conventional hydrogen peroxide sterilization. Moreover, neither chemical agents nor a sterilization gas is necessary and hence it is also low cost.

Even when compared to sterilization by high-pressure steam etc., the air plasma treatment is predominant. The microwave plasma is generated by the absorption of microwave energy under ambient conditions. The microwave plasma can give high energy to a small area without generating a large amount heat and high density and high energy active species at low temperature are suitable for medical sterilization. High-energy electrons in the microwave plasma enable the production of active species such as nitrogen oxides using air, which cannot be available in a conventional plasma sterilizer. The nitrogen oxides are expected to achieve rapid sterilization compared with other plasma sterilization

* Corresponding author. Interdisciplinary Graduate School of Engineering Sciences, Kyushu University 6-1 Kasuga-koen Kasuga-shi, Fukuoka 816-8580, Japan. Tel.: +81 92 583 7649; fax: +81 92 5718894.

E-mail address: itarashiki@saraya.com (T. Itarashiki).

methods such as the RF plasma sterilizer [10]. The NO radical produced in the air plasma has a deoxidizing characteristic for organic compounds, and proteins on the surface of microorganisms are deoxidized and decomposed by NO radical irradiation. The NO radical addition to protein molecules on the surface of microorganisms changes the original function of proteins, and the microorganisms are inactivated [4,5]. RF discharge in a tiny area with high energy generates a large amount of heat owing to the collisions between the ions and neutral particles but the heat production by the microwave discharge in the small area is relatively small, because of limited movement of ions in the microwave electric field. However, in this research, in order to realize sterilization at low temperatures, the temperature in the vacuum chamber was maintained at 60 °C or less. The vacuum chamber internal pressure and microwave power output and the vacuum chamber size are important parameters. In this study, the optimum conditions for low temperature microwave plasma sterilization were determined.

2. Experimental procedure

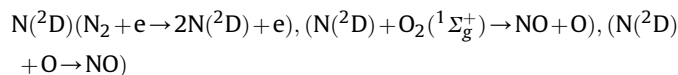
The plasma source used in this experiment is the microwave torch plasma, shown in Fig. 1, in order to obtain radicals with high concentration originating from the air discharge. The experimental device consists of a microwave power supply, a waveguide, glass tube with microwave antenna, a vacuum vessel, and a vacuum pump, which allow plasma production. Plasma is generated within the glass tube located in the waveguide. The generated plasma is sent into a treatment region. The bacilli (BI) were 3 cm below the opening edge of the torch. The frequency of the microwave power supply was 2.45 GHz. The inside diameter of the glass tube of an antenna was 5 mm diameter. At the antenna, the slit of the half-wave length (approximately 6 cm) of the microwave is located. The size of the vacuum chamber is approximately 17.5 L. The electron density of the plasma plume ejected from the torch opening edge is measured using a Langmuir probe with a cylindrical electrode of 1 mm dia. and 4 mm in length. The vacuum vessel is evacuated to several Pa. The gas for plasma production flows into the vacuum vessel to a pressure of several hundred Pa. The plasma is generated by absorption of microwaves by an electrode wound around a silica tube. The plasma gas acts on the object to be sterilized before it is placed into the vacuum vessel. The plasma gas is made to flow in the vacuum vessel. By colliding with an atom or a molecule and high-energy electrons, active species (radicals) are produced, and bacilli are annihilated via chemical reaction with the radicals. The ultraviolet rays emitted from an excited atom with high-energy electrons also damage the bacterial DNA. In addition, charged particles, such as electrons and ions

collide directly with the bacteria and annihilate them. Sterilization is thus a method for annihilating all microbes present. The time required to decrease the microbes in a sterilization chamber to 1/10th the original number is called the D value of the sterilization method. In order to determine the D value, BI comprising *Bacillus subtilis* var. *natto*. is utilized. A biological indicator (BI) is used to evaluate the sterilization efficacy. The BI contains bacterial spores of *Geobacillus stearothermophilus* ATCC7953, which shows the heat resistance [11,12]. This is a regular method of cultivating after the end of sterilization and checking the extinction of bacterial spores.

3. Experimental results and discussion

3.1. Generation of active species

Fig. 2 shows a photograph of the after-glow of the microwave torch plasma, taken from the view port located on the side of the plasma treatment chamber. Plasma with a yellowish color and high spatial uniformity is spread over the whole treatment region. The Langmuir probe measurements indicate that the charged particles have disappeared from the discharge region to the opening edge of the torch because of the higher pressure of several ten Pa and the distance between the discharge region and the opening edge [13]. The origin of the light emission is NO-radicals produced outside the torch by metastable nitrogen atoms and oxygen atoms or molecules.



Metastable nitrogen atoms $\text{N}(^2\text{D})$ are produced in the discharge region and transported outside the torch owing to their long lifetime. The electron density at the position 7 mm below the opening edge of the torch, where the yellowish light emission is observed, is lower than 10^5 cm^{-3} (the detection threshold of the Langmuir probe system). This result implies that the yellowish light originates from the NO radical generated from the combination between a metastable nitrogen atom $\text{N}(^2\text{D})$ and oxygen atom or molecule outside the torch. Since it is presumed that light emission intensity is proportional to the sterilization performance, light emission spectra in the vacuum vessel were measured by varying the pressure and microwave input power, as shown in Fig. 3. The 400–800 nm regime appears in the light emission spectra, and the height of the peak increases with increasing microwave power. This peak is completely different from the peaks in the spectra of air and

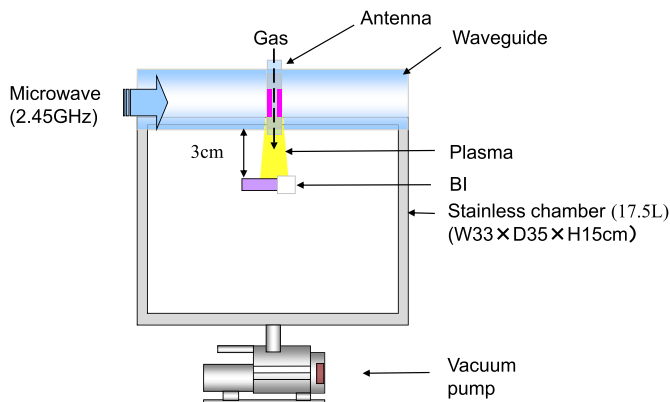


Fig. 1. A schematic view of the generation equipment for microwave plasma.

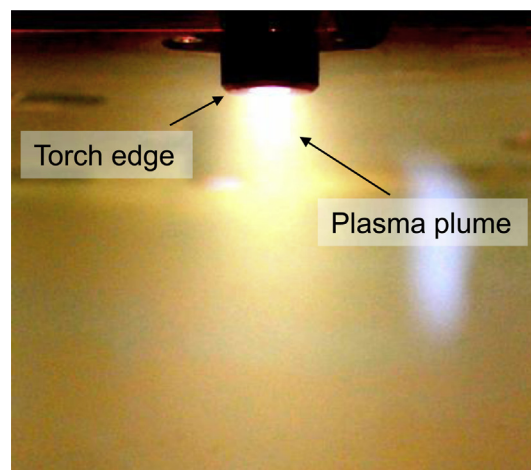


Fig. 2. The plasma is emitted from the antenna apical portion.

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