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Sterilization using a microwave-induced argon plasma system at atmospheric pressure

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The use of microwave plasma for sterilization is relatively new. The advantages of this method are the relatively low temperature, time-savings and its nontoxic nature, in contrast to traditional methods such as heat and gas treatment, and radiation. This study investigated the sterilization effects of microwave-induced argon plasma at atmospheric pressure on materials contaminated with various microorganisms, such as bacteria and fungi. A low-cost and reliable 2.45 GHz, waveguide-based applicator was designed to generate microwave plasma at atmospheric pressure. This system consisted of a 1 kW magnetron power supply, a WR-284 copper waveguide, an applicator including a tuning section, and a nozzle section. Six bacterial and fungal strains were used for the sterilization test. The results showed that regardless of the strain, all the bacteria used in this study were fully sterilized within 20 seconds and all the fungi were sterilized within 1 second. These results show that this sterilization method is easy to use, requires significantly less time than the other traditional methods and established plasma sterilization methods, and it is nontoxic. It can be used in the field of sterilization in medical and dental clinics as well as in laboratory settings. [DOI: 10.1063/1.1613655]

I. INTRODUCTION

Sterilization is based on either a physical or a chemical process that destroys or eliminates microorganisms, or both.[1,2] Traditional methods for sterilization include autoclaving (moist heat), ovens (dry heat), chemical such as ethylene oxide (EtO), and radiation (gamma rays), which are dependable and well understood. However, all of these methods have their advantages as well as their disadvantages. Heat treatment methods may not be suitable for low-melting point materials such as polymer-based materials.[1] An EtO treatment is highly toxic, which can be absorbed in plastics[3–6] Radiation may also cause the material to undergo undesirable changes during sterilization.[7–9] For these reasons, a more rapid and less damaging method of sterilizing various materials is needed.[10]

A new sterilization method in the field of protection and conservation of materials from microorganisms is plasma treatment. Plasma treatment is a well-established technique in a number of processes, e.g., plasma cleaning, etching, and coatings.[11]

Sterilization by plasma is an alternative to conventional sterilization methods. Moreover, the advantages of microwave plasma sterilization include the possibility of sterilization at a relatively low temperature, preserving the integrity of polymer-based materials,[1] and it is safe as opposed to EtO.[2,12] Moreover, it is not only capable of killing bacteria and viruses, but it is also capable of removing the dead bacteria and viruses (pyrogens) from the surface of the objects being sterilized.[1,13]

This paper describes a new method, microwave-induced argon plasma at atmospheric pressure for the sterilization of microorganisms, and presents some experimental results regarding the sterilization of some selected bacteria and fungi.

II. MATERIALS AND METHODS

A. Microwave-induced argon plasma system setup

Figure 1 shows a schematic diagram (a) and a 2.45 GHz, waveguide-based, microwave-induced argon plasma system

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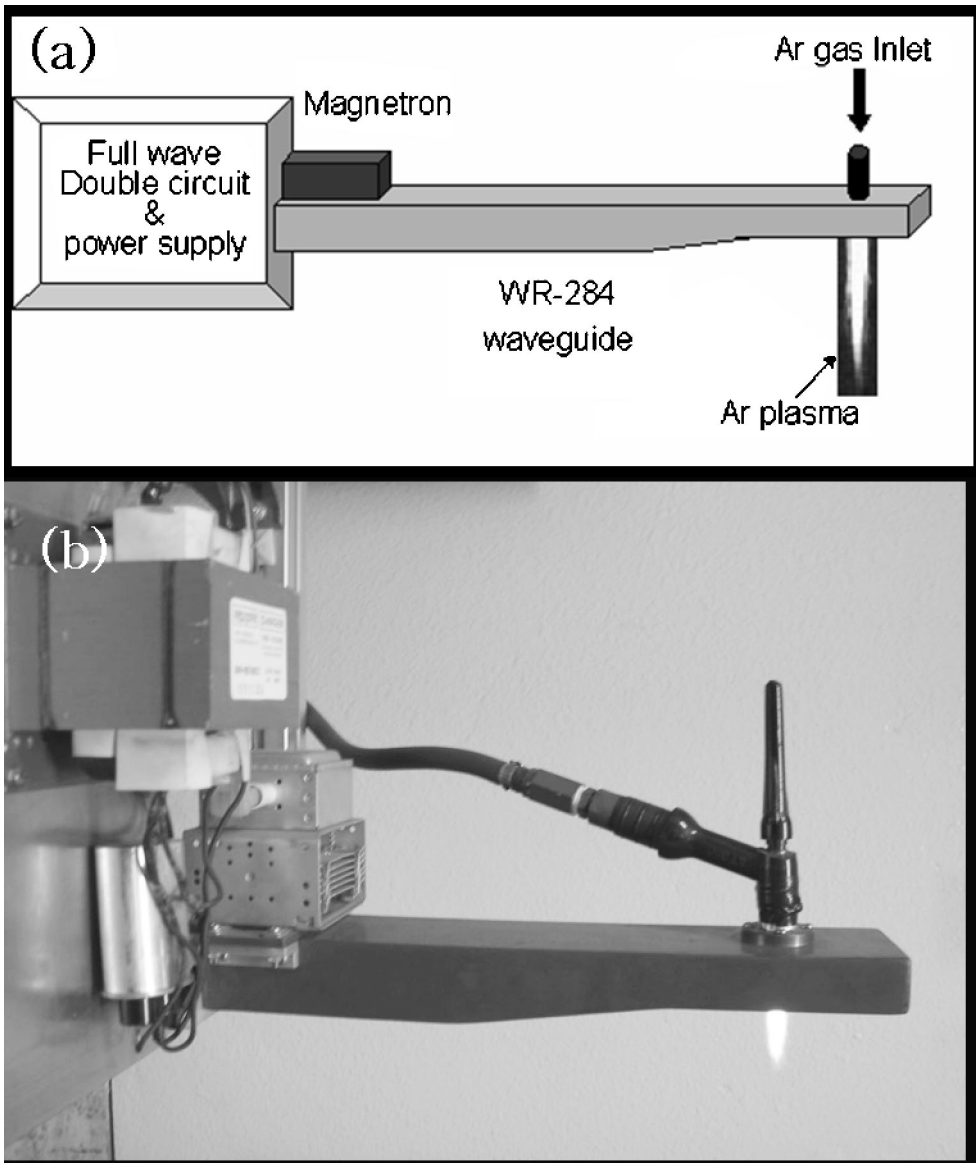

FIG. 1. Microwave-induced argon plasma system. (a) Schematic diagram. (b) A 1 kW, 2.45 GHz microwave-induced argon plasma system.

to generate plasma at atmospheric pressure. This system consists of a 1 kW magnetron power supply that is commonly used in a microwave oven, a WR-284 copper waveguide, with internal cross section dimensions of 72 mm$\times$34 mm, an applicator including a tuning section, which is needed to reduce the reflected power, and a nozzle section made of quartz, which is commonly used in a Tungsten Inert Gas (TIG) welding machine. The WR-284 waveguide was tapered off to increase the electric field strength and to minimize the reflected wave in the region of interest. For impedance matching, the location of magnetron antenna was calculated using a High Frequency Structure Simulator (HFSS) code simulation[14] as shown in Fig. 2. The gas nozzle sits inside the cavity directly in the waveguide as shown in Fig. 1. The distance from the waveguide center to the open end of the nozzle was several centimeters. The nozzle was located a quarter λ away from the shorted end of the waveguide where the electric field intensity was at its maximum. This location (l) was calculated using the following equation:

$$l = \frac{\lambda}{4\sqrt{1+(\lambda/a)^2}},$$

where l is the distance from the shorted end of the waveguide, a is the wide of the waveguide, and λ is the wavelength of the electromagnetic wave in the waveguide, and the distribution of the electric field at this location was confirmed by an HFSS code simulation, in which the electric field intensity around the nozzle was calculated, as shown in Fig. 3. The plasma generated at the end of the nozzle was formed by an interaction between the high electrical field, which is generated by the microwave power, the waveguide aperture and the gas nozzle (Fig. 4). Argon was used as the working gas for this plasma system, which was chosen for its inertness, and the gas flow rate is approximately 100 liters per minute (l pm) at 4 kgf/cm^2.

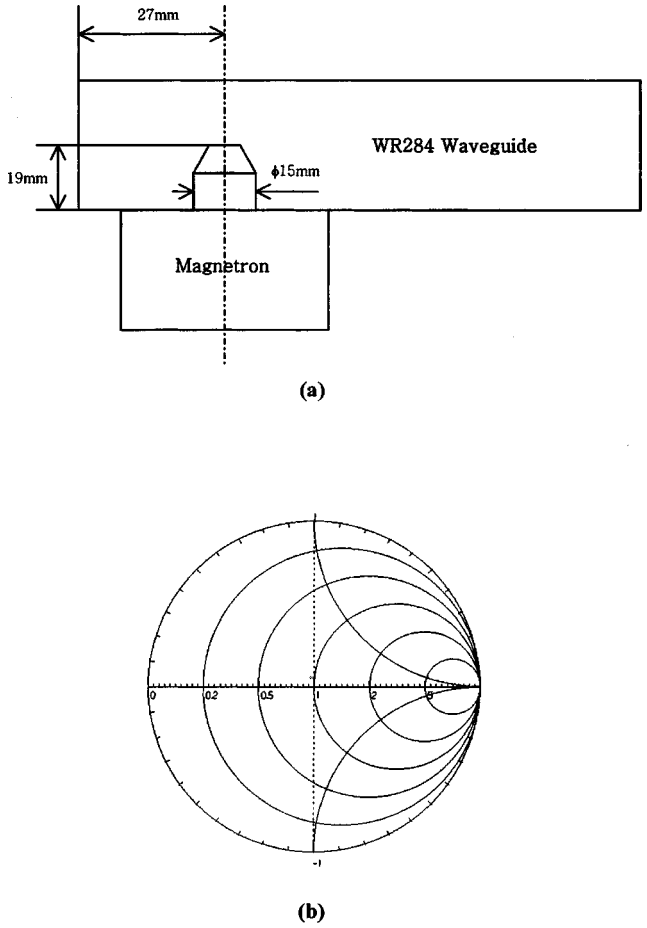

FIG. 2. The impedance matching to transfer efficiently the electromagnetic wave generated by the magnetron. (a) The impedance matched location of the magnetron antenna. (b) Smith chart calculated by the HFSS code simulation.

B. Microorganisms

The four bacterial strains used in this study, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 11775, *Pseudomonas aeruginosa* ATCC 9027, and *Salmonella typhimurium* ATCC 14028, were obtained from the American Type Culture Collection (Rockville, MD, USA), and were maintained on standard methods agar (Difco, Detroit, USA) slants at 5–6 °C. The cultures were kept at 35 °C for three days.

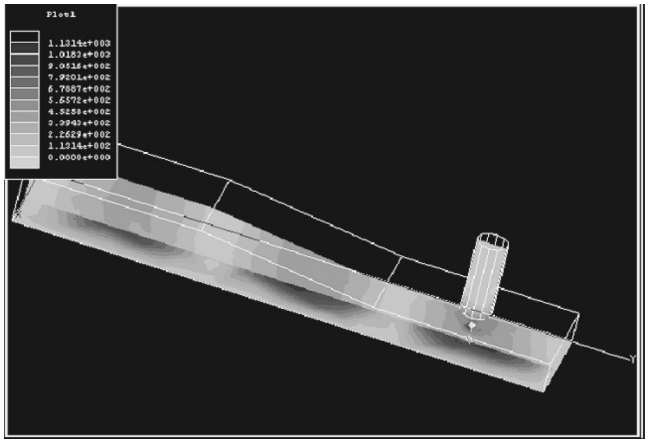

FIG. 3. The distribution of the electric field at the waveguide calculated by the HFSS code simulation.

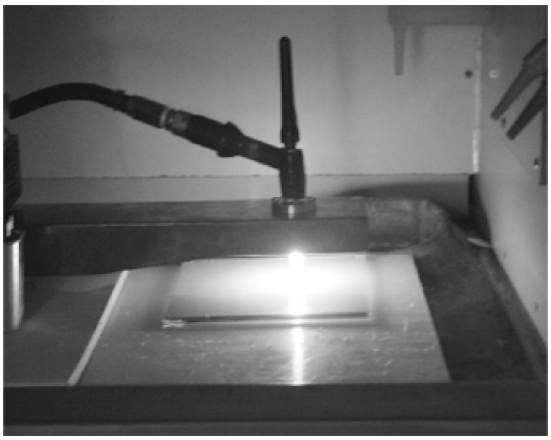

FIG. 4. Microwave-induced argon plasma generated at atmospheric pressure. The plasma generated at the end of the nozzle is formed by an interaction the high electrical field, generated by the microwave power, with the waveguide aperture and the gas nozzle.

Two fungal strains, *Aspergillus niger* Yonsei Medical Center (YMC) 0100 and *Penicillium citrinum* YMC 0253, which were identified by their mycological characteristics,[15] were isolated from indoor dust and air and used as stock cultures. All fungal strains were maintained on potato-dextrose agar (Difco, Detroit, USA) slants supplemented with 30 μg/l chloramphenicol (Wako Pure Chemical, Tokyo, Japan) in order to suppress bacterial growth,[16] and the cultures were kept at 25 °C for seven days.

C. Sterilization test using microwave-induced argon plasma at atmospheric pressure

For the sterilization test, the six bacterial and fungal strains were suspended in 0.9% saline. The suspensions of the bacterial and fungal spores were inoculated on to sterilized filter papers (10 mm×10 mm×0.75 mm) in Petri dishes, and allowed to dry at room temperature for 1 hour. Prior to the sterilization test, the filter papers inoculated with bacteria or fungi were removed from the Petri dishes and placed in front of a nozzle in the plasma. All the strains were exposed to the plasma for 1, 2, 3, 4, 5, 10, 20, and 30 s. After plasma treatment, the filter papers were transferred into a screw cap tube containing 2 ml of 0.9% saline, and shaken for 60 seconds. The strains in the saline were spread over a standard plate agar for bacteria and on potato-dextrose agar for fungi. The number of colonies was counted after one day of incubation at 37 °C for the bacteria, and after seven days of incubation at 25 °C for the fungi.

Bacillus subtilis and *Penicillium citrinum* were inoculated on slide glasses coated with poly-L-lysine to promote the adhesion of the strains during the plasma treatment in order to observe the morphologies of the strains using a scanning electron microscope (SEM). The slides were then, subjected to the plasma as previously described, for 5, 10, and 20 seconds for bacteria and 1, 5, and 10 seconds for fungi. The slide glasses, both treated and untreated, were

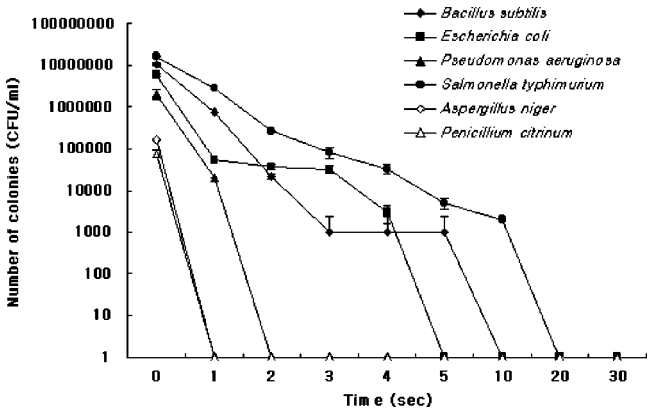

FIG. 5. Sterilization effects by the microwave-induced argon plasma at atmospheric pressure.

coated with an ultra-thin layer of gold/Pt by ion sputter (E1010, HITACHI, Tokyo, Japan), and the morphologies observed by a SEM (S-800, HITACHI, Tokyo, Japan).

In order to determine the effect of a microwave-only treatment on the microorganisms, a sterilization test using a commercial microwave oven, with a 1 kW, 2.45 GHz magnetron power supply, was carried out using the same method as described above for 10, 20, 30, and 60 seconds.

D. Detection of the intensity of generated UV light and the temperature of surface treated with plasma

In order to evaluate the effect of the UV generated by microwave-induced argon plasma at atmospheric pressure, the intensity of generated UV light was measured indirectly using a radiometer/photometer (IL1400A, International Light, Inc., Newburyport, MA, USA) with a solar blind vacuum photodiode. In addition, the temperature of surface treated with plasma was measured directly using a thermo label (Thermo label 5E, Nichiyu Giken Kogyo Co., Ltd., Osaka, Japan) for 1, 5, 10, and 30 seconds to evaluate the effect of the temperature.

III. EXPERIMENTAL RESULTS

The sterilization effects of microwave-induced argon plasma at atmospheric pressure on bacteria and fungi are shown in Fig. 5. Although *Pseudomonas aeruginosa* bacteria were found to be the most sensitive strain to the plasma, all the bacteria used in this study were fully sterilized in less than 20 seconds. The number of colonies and the plasma treatment time showed time-dependent relationship in accordance with the strains. SEM observations confirmed the sterilization effect of the spores by microwave-induced argon plasma. Figure 6 shows the SEM images of *Bacillus subtilis*. The untreated *Bacillus subtilis* cells [Fig. 6(a)] were ellipsoidal, with average dimensions of 1.2 μm$\times$0.6 μm, while the plasma treated spores [Figs. 6(b), 6(c), and 6(d)] were smaller than the controls, and their cell membranes were damaged and ruptured [Figs. 6(b), 6(c)]. Therefore, the cellular contents were released into the surrounding surface.

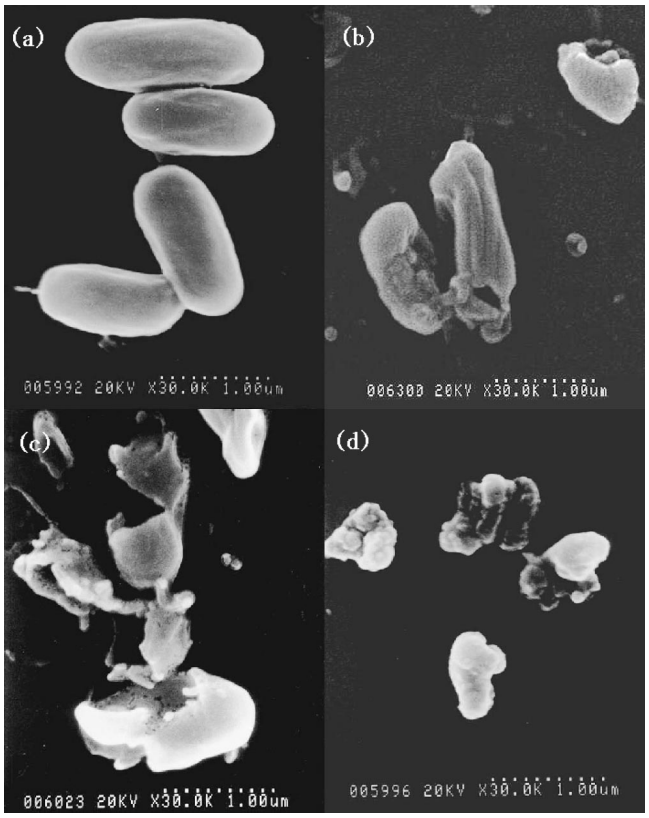

FIG. 6. Scanning electron microscope (SEM) photographs of *Bacillus subtilis*: (a) untreated control; (b) Spores exposed to $t=5$ s, (c) $t=10$ s, and (d) $t=20$ s of microwave-induced argon plasma at atmospheric pressure.

After 20 seconds exposure, the *Bacillus subtilis* cells were reduced to smaller structures and microscopic debris, as shown in Fig. 6(d).

In the case of fungi, a much shorter plasma treatment time than what was required for bacteria was needed, as shown in Fig. 5. All the fungi were fully sterilized in less than 1 second regardless of the strain. Figure 7 shows SEM images of *Penicillium citrinum*. Figure 7(a) shows normal spores, which have a globular shape, with no plasma exposure, while Figs. 7(b), 7(c), and 7(d) show the effects of 1, 5, and 10 seconds of plasma exposure. The *Penicillium citrinum* spores were rapidly damaged. There were holes in the cell walls, a significant reduction in size, as well as a transformed and amorphous morphology [Figs. 7(b) and 7(c)]. Therefore, the *Penicillium citrinum* spores were also reduced to smaller structures and microscopic debris after 10 seconds exposure [Fig. 7(d)].

Figure 8 shows the effects of the microwave oven on the bacteria and fungi. In these results, the microwave-only treatment exhibited no effect on bacterial and fungal viability in up to 1 minute.

The UV intensity generated by the plasma using a radiometer/photometer ranged from 65 mW/cm^2 (minimum) to 94 mW/cm^2 (maximum) at a wavelength of 254 nm. In addition, temperature of surfaces treated with the plasma, for 1, 5, 10, and 30 seconds were approximately 75, 105, 115, and 130 °C, respectively.

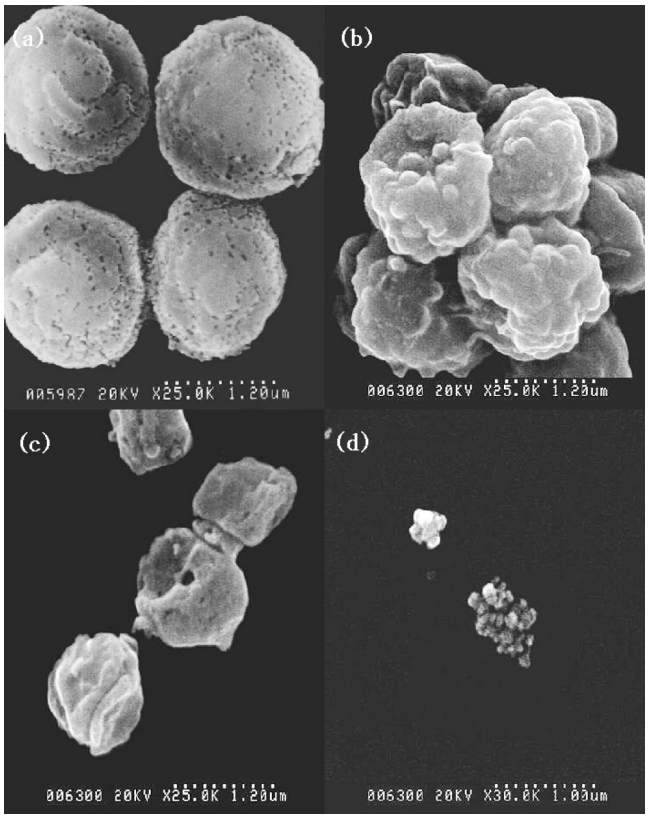

FIG. 7. Scanning electron microscope (SEM) photographs of *Penicillium citrinum*: (a) untreated control; (b) spores exposed to $t=1$ s, (c) $t=5$ s, and (d) $t=10$ s of microwave-induced argon plasma at atmospheric pressure.

IV. DISCUSSION

Generally, sterilization is based on either a physical or chemical process, or both, and the microorganisms can be inactivated by heat,[17] radiation,[17,18] or chemical treatment.[3] Inactivating bacteria and fungi from materials is important, but the removal of killed bacteria and fungi or their debris from the materials is also important.[1] Therefore, it has been suggested that various plasmas, including microwave plasma, can be used for sterilizing microorganisms.[1,2,10,12]

Microwave plasmas are generated without electrodes.[19–23] Thus, the plasma can be excited in various applications, and can provide a stable, continuous plasma stream over a large range of gas pressures.[24] The microwave plasma generated by an atmospheric pressure gas discharge is a source of electrons, ions, excited atoms and molecules, active free radicals, and UV radiation. These factors qualify microwave plasma as a unique sterilization agent for various applications[25] and materials, including the field of medical and biological materials.

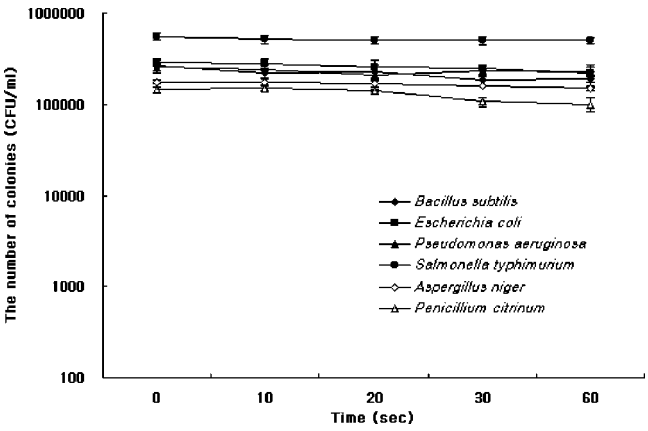

FIG. 8. Sterilization effects using a commercial microwave oven.

In this study, the sterilization effects of microwave-induced argon plasma at atmospheric pressure on the microorganisms were evaluated. All the bacteria used were fully sterilized in less than 20 seconds, although the survival curves were different according to the type of microorganism. All fungi were perfectly sterilized in less than 1 second regardless of the strain, as shown in Fig. 5. These results assumed that there is a great difference in the structure of the cell wall,[26,27] as well as a great difference in the sensitivity between the bacteria and fungi to plasma. Reactive species and UV light generated by the plasma in fungi can diffuse through an otherwise chemically and physically robust membrane more rapidly in fungi than bacteria, and directly react with the biomaterials inside the cell. Therefore, fungi require significantly less time than bacteria to be sterilized in the plasma. These results demonstrate the possibility of using microwave-induced argon plasma at atmospheric pressure to sterilize materials contaminated with microorganisms.

In general, there are three basic mechanisms. These are DNA destruction by UV irradiation, the erosion of the microorganism through intrinsic photodesorption, and etching (eventually enhanced by UV radiation) in the plasma.[2,28–30] These processes were confirmed in this study using microwave-induced argon plasma at atmospheric pressure.

UV radiation can penetrate the cell walls of microorganisms causing the disruption of unsaturated bonds, particularly the purine and pyrimidine components of the nucleoproteins.[1,24] The generated UV and activated free radicals in the microwave plasma first weaken the cell wall of the microorganisms by their reaction with the hydrocarbon bonds. As the process continues, the microwave plasma removes the outer layer of the microorganisms.[1] At this point, either the bacterial cell wall bursts or its internal structure is destroyed by the activated free radicals and UV radiation.

In this study, the intensity of UV light generated by the plasma ranged from 65 mW/cm^2 (minimum) to 94 mW/cm^2 (maximum) at a wavelength of 254 nm, which killed the microorganisms. Figures 6 and 7 show SEM images of the *Bacillus subtilis* and *Penicillium citrinu* spores that were ruptured and damaged by the microwave plasma. The figures show that there was a relatively high level of UV emission in the microwave-induced argon plasma, and that the UV radiation generated was involved in sterilizing the microorganisms. Furthermore, a relatively high level of UV light generated by the plasma enhanced the etching process.

Another sterilization process of microwave plasma, and one similar to plasma etching, is the erosion of the microorganisms through etching to form volatile compounds, as a result of slow combustion using oxygen atoms or radicals emanating from the plasma. This can be seen in Fig. 6(d) and Figs. 7(c) and 7(d). These SEM images show a significant

reduction in size, and exhibit transformed and amorphous morphologies. Therefore, the spores of the microorganisms were reduced to smaller structures and microscopic debris after plasma exposure. This result demonstrated that a strong etching process of the plasma caused the sterilizing effect on the microorganisms, when the microorganisms were exposed to this microwave plasma at atmospheric pressure.

The effect of the microwave-only treatment was evaluated by a sterilization test using a commercial microwave oven. The effectiveness of the microwaves in sterilizing the microorganisms is well established,[31–33] although a larger exposure time—of one or more minutes—is normally required. In this study, a microwave-only treatment of up to 1 minute had no sterilization effect, as shown in Fig. 8. This result shows that the sterilization effects of microwave-induced argon plasma are due to the plasma, and not the microwave.

Although, the temperatures of the surface treated with plasma for 1, 5, 10, and 30 seconds were measured from 75 to 130 °C, the effect of heat was not investigated in this study since it is assumed that the heat generated by the plasma was dry heat. Generally, dry heat takes approximately 120, 60, and 30 minutes at 160, 170, and 180 °C to get complete sterilization.[25,27,28,36,37] Therefore, it requires higher temperatures and longer times than our study for sterilization.

V. CONCLUSION

This study confirmed that the sterilization effects of microwave-induced argon plasma at atmospheric pressure on the microorganisms caused by the generation of free radicals, and UV light, as well as the etching process. All the bacteria and fungi tested showed time-dependent effects in the sterilization test using microwave-induced argon plasma at atmospheric pressure, with the fungi requiring much less exposure time than the bacteria. Furthermore, the microwave plasma system required much less exposure time than what has been reported from most published systems,[1,2,10,12,23–35] because of the high plasma density, the large number of free radicals and the strong intensity of generated UV light.

These results suggest that this sterilization method is easy to use, requires significantly less exposure time than other methods, such as traditional methods and different established plasma sterilization methods. In addition, it is nontoxic. Certainly, more tests and in-depth studies of this process are needed. Nevertheless, this study represents the first step in showing that microwave-induced argon plasma at atmospheric pressure is a powerful sterilization tool for materials contaminated with microorganisms.

ACKNOWLEDGMENT

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