Development of catalyst materials being effective for microwave sterilization

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Received 4 March 2005; revised 15 July 2005; accepted 18 July 2005
Available online 3 November 2005

Abstract

Recently, airborne virus infections have emerged as some of the most challenging medical problems. To prevent the threat of infection, the processes of sterilization have been studied widely. Microwave sterilization has many advantages in comparison with conventional methods. It is able to raise the temperature of a material in a short time and selectively heat the material. This results in the reduction of usage and the rapid completion of sterilization. We developed a novel microwave sterilization system that can raise the temperature in quite a short time using a lower microwave power (100 W). Filters made with Kao wool (Al₂O₃) were coated with TiO₂ (anatase) by sol–gel method and used to trap microorganisms. In addition, these filters were coated with Pt or Ag by impregnated method. We also prepared a Tyranno-fiber textile filter and a honeycomb SiC filter. Two microorganisms, *Bacillus subtilis* ATCC 9372 and *Bacillus stearothermophilus* ATCC 7953, were used in this experiment, where either microorganism was loaded onto a filter. After irradiation, filters loaded with *B. subtilis* and *B. stearothermophilus* were incubated for 48 h in a TSB medium (Bacto™ Tryptic Soy Broth, Becton, Dickinson and company sparks, MD) at 37 and 56 °C, respectively. *B. subtilis* and *B. stearothermophilus* loaded on to Ag-impregnated filters were sterilized in 30 and 15 s, respectively. The Tyranno-fiber textile filter and the honeycomb SiC also filter showed effective microwave sterilization. These results showed that this system could sterilize *B. subtilis* and *B. stearothermophilus* in quite a short time and that microwave absorbable materials are effective as microwave sterilization filters.

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Keywords: Catalyst materials; Filter; Microorganisms; Microwave sterilization

1. Introduction

In the last several years, various influenza viruses such as avian flu [1] and SARS virus [2] have emerged as some of the most challenging medical problems. As these viruses are airborne, a good air purification system is required at places where people gather, such as schools, offices, theaters, hospitals, restaurants and assembly halls. To prevent such threat of infection, many types of sterilization processes have been studied widely. Such processes include: heat sterilization [3], ozone gas sterilization [4–5], plasma sterilization [6–7], UV light sterilization [8], microwave sterilization [9–14] and so on. In heat sterilization, the whole interior of the system is heated. This consumes a lot of energy and time. Ozone gas sterilization also has some difficulties. Ozone, which is generated by plasma etc., consumes some chemical or physical reaction. In order to improve the sterilization ability, the density of ozone should be elevated. However, this poses a problem, as ozone is toxic. Plasma sterilization is a good sterilization system, however, stabilizing the electric discharge of the airflow system is difficult. Hence, this system is impractical as an air purification system. UV sterilization is widely used in hospitals and laboratories. This is because it is compact, possesses a high sterilization effect and is easy to use. But UV irradiation can sterilize only the radiated area. This effect is called the shadow effect. Because of these problems, these sterilization methods are not practical for air purification systems. Amongst all the sterilization methods, microwave sterilization is the most feasible. It has many advantages in comparison with conventional methods. It is able to heat a material directly, rapidly and selectively, resulting in a reduction of power usage. In addition, microwave sterilization can sterilize not only the surface of a material, but also the interior. Because the microwave is irradiated from all angles, it can sterilize the micrograms effectively. Based on these advantages, microwave sterilization is applied widely in industrial food processing, hospital waste processing and so on.

However, microwave sterilization in the air has following problems:
How to trap bacteria, viruses etc.
How to obtain effective sterilization by low power microwave irradiation
How to ensure the effective sterilization in a short time

In order to solve these problems, we tried to develop a novel sterilization method in which we could raise the temperature of a material in quite a short time using a lower microwave power (100 W). In our previous experiments, catalyst materials containing TiO₂ (anatase) showed an excellent sterilization effect when used in microwave sterilization [15]. We concluded that the most activated catalysts are Pt and Ag loaded on to a TiO₂–SiO₂ and/or Al₂O₃ mixed carrier. These metal films played an important role in this sterilization system. Based on our previous experiments, filters made with Kao wool (Al₂O₃) were coated with TiO₂ (anatase) by the sol–gel method and used to trap microorganisms. These filters were then coated with two kinds of metals (Pt, Ag) by the impregnated method. We also prepared Tyranno-fiber textile and honeycomb SiC filters. Tyranno-textile is composed of Si–C–Ti–O system fibers. Honeycomb SiC is composed of SiC. As these materials contain carbon and SiC, they are good absorbers for microwaves. We chose two microorganisms, *Bacillus subtilis* ATCC 9372 and *Bacillus stearothermophilus* ATCC 7953, because these microorganisms possess heat-resistance property, have strong vitality, and are not toxic. The microorganisms were loaded onto filters. After microwave irradiation, filters loaded with *B. subtilis* and *B. stearothermophilus* were incubated for 48 h in a TSB medium at 37 and 56 °C, respectively. *B. subtilis* and *B. stearothermophilus* loaded on to Ag-impregnated filters could be sterilized by microwave irradiation in 30 and 15 s, respectively. The Tyranno-fiber textile and the honeycomb SiC filter showed effective microwave sterilization, too. These results showed that this system could sterilize *B. subtilis* and *B. stearothermophilus* in quite a short time.

2. Experimental

2.1. Preparation of catalyst filters

We prepared Kao wool (alumina ceramic fiber) as filters to trap the microorganisms. They were coated with TiO₂ (anatase) by sol–gel method. The Kao wool (1 mm thick) was cut into squares with a length of 18 mm. They were then washed with acetone, and dried at room temperature. Ethanol was put in a beaker, and a very small amount of acetyl acetone was added in order to prevent the progress of rapid hydrolysis and to hinder the crystal growth of TiO₂. Alumina sheets were completely submerged in this liquid. Then, titanium tetraisopropoxide was added to the beaker under shaking in order to prevent rapid hardening, caused by hydrolysis and the immediate condensation reaction. The beaker was kept at room temperature under shaking. The filters were taken out after 18 h, and placed on a ceramic board, and calcined for 2 h at 600 °C. After calcination, each of the filters was submerged into a platinum acid (H₂PtCl₆·6H₂O) or silver nitrate (AgNO₃) water solution (200 ml) in order to impregnate the filters with these metal ions at room temperature. The density of the metal ion solution was 1.7553 × 10⁻⁵ mol/ml. After stirring for 30 min, the filters were taken out of the water solution and dried at room temperature. Finally, the filters were sintered at 300 °C for 1 h in a hydrogen reduction system in order to remove oxygen from the surface of the metal ions. Fig. 1 shows the catalyst filters. These filters were irradiated by UV for 20 min to sterilize any contamination, and kept in a germfree laboratory dish.

We prepared a Tyranno-fiber textile (Ube Industries, Ltd) and a honeycomb SiC (TYK Co., Ltd) as filters by the following methods. Tyranno-fiber textile was cut into squares with a length of 18 mm, and washed with acetone to remove the bonding resin. To prevent the loosening of the edges (in Fig. 2), the Tyranno-fiber textile was hardened by ceramic resin at room temperature. Honeycomb SiC (Fig. 3) was cut into lengths of 5–10 mm, and arranged into a shape of a circle and connected by a ceramic resin. They were irradiated by UV for...
20 min in order to sterilize any contamination, and kept in a germfree laboratory dish.

2.2. Preparation of microorganisms

We tested two microorganism, *B. subtilis* ATCC 9372 Attest™ 1264 and *B. stearothermophilus* ATCC 7953 Attest™ 1262 (3 M Company). We used a biological indicator as the source for microorganisms. The filter paper was picked-up from this BI on which the microorganism was adsorbed.

The filter paper was located on the microorganism supporter in the microwave sterilization apparatus. Each filter paper contained *B. subtilis* (3.5 × 10⁶ CFU) or *B. stearothermophilus* (5.2 × 10⁵ CFU).

2.3. Microwave sterilization system

Fig. 4 shows the experimental setup for the sterilization by microwave irradiation. A catalyst filter was placed before the decline part of the quartz tube. The quartz tube is a double structure, where the inside of the quartz squeezed the filter to support it tightly. A HEPA-CAP filter was connected to the apparatus to trap dust. Air was inhaled from the inlet, and allowed to pass through the PEPA-CAP and catalyst filter, and exhausted from the vent by an exhaust fan. Airflow was controlled by flow control valve. A microorganism supporter attached with *B. subtilis* or *B. stearothermophilus*, was supported by a wire which was fixed to a vibrator. When the vibrator was switched on, the microorganisms were released from the microorganism supporter, and they were transported to the filter and, trapped by the filter. The quartz tube was set at about 1/4 wavelength from the end of the microwave waveguide. The microwave power was concentrated at this point, and adjusted at 100 W without reflected microwave power.

2.4. Operation of microwave sterilization

Before carrying out the experiments, the inside of the quartz tube was sterilized with 75% ethanol. The filter was set up inside the quartz tube. The quartz tube was connected to the apparatus as seen in Fig. 4. The microorganism supporter was clipped to the wires. The exhaust fan and the vibrator were started simultaneously and operated for 1 min, keeping airflow at 10 L/min. Then, they were stopped simultaneously. Microwaves were irradiated for various time intervals. We checked to ensure that a leak in microwave power not to damage the operator during irradiating microwave. After irradiation, the filter was taken out from the apparatus, and dipped into a test tube containing 2 ml of TSB medium, and incubated at 37 and 56 °C for *B. subtilis* and *B. stearothermophilus*, respectively. After 48 h incubation, the sterilization effect was evaluated by the turbidity of each TSB medium.
3. Results and discussion

3.1. Evaluation of catalyst filters

We performed surface and structure analyses of the catalyst filters. Fig. 5 shows the digital microscope image of the catalyst filter by VHX-200 (Keyence). This alumina filter was coated with TiO₂ by sol–gel method. When a filter with minute pores was used the air stream was bad. While if it was too sparse, all microorganisms would go through it. This filter possessing appropriate holes was best for an air purification system.

The crystal phase of anatase in the filter was measured by XRD (X-ray diffraction) (Rigaku, RAD-C). Generally, the anatase phase of TiO₂ is well known as an activated catalyst. We hoped that the OH* radical produced on the surface of anatase by the catalysis reaction would have a strong oxidative effect. This phenomenon contributes to the elevation of the sterilization ability of spores [16]. The anatase crystal of TiO₂ synthesized by the sol–gel method is well known as an activated catalyst [17]. The catalytic activity of anatase phase is kept in the region of low temperature, but the appearance of the rutile phase causes it to lose activity. Fig. 6 shows the anatase phase of the filter sintered at 600 °C. The rutile or brookite peaks did not appear in the spectrum.

3.2. Evaluation of microwave sterilization

The sterility was assessed by the turbidity of the TBS medium after 48 h incubation at 37 or 56 °C. Fig. 9 shows a typical example of the experimental result: the control test-tube (muddy broth) for 0 s, vice versa the experimental test-tube (clear broth) for 15, 30, 60, 120 and 150 s. Table 1 shows the comparison of the sterilization effect of microwave irradiation for Kao wool filter impregnated with Pt or Ag. B. subtilis on the Ag-coated filter was completely sterilized within 30 s. This was a superior result. B. subtilis on the Pt-coated filter was also sterilized in a short time in comparison with the non-coated filter. The results show that a metal-coated filter is extremely effective in microwave sterilization. The metal thin film
(less than 1 μm) strongly absorbs electromagnetic waves. Because the metal thin film on the filter was heated selectively by microwave, the surface of the filter was heated quickly. As for **B. stearothermophilus**, all of the filters were completely sterilized within 15 s. While **B. stearothermophilus** is generally considered to be thermostable in comparison with **B. subtilis**, **B. stearothermophilus** was sterilized in shorter time than **B. subtilis** by microwave irradiation. Other factors may have affected **B. stearothermophilus** sterilization. We suppose that OH* radical generated on the surface of anatase by microwave irradiation, and sterilized **B. stearothermophilus**. Similarly, we tested other materials like Tyranno-fiber textile and Honeycomb SiC. Table 2 shows the results of the sterilization effect of Tyranno-fiber textile and honeycomb SiC filters irradiated with microwaves. Tyranno-fiber textile and honeycomb SiC could completely sterilize both microorganisms within 30 s. These results suggest that microwave absorbable materials are effective as microwave sterilization filters.

### 4. Conclusion

We showed that microwave heating is important in microwave sterilization technique. In addition, the sterilization effect varied with the kind of coated metal. The sterilization effect of the filter coated with silver was excellent. **B. stearothermophilus** on all of the filters were completely sterilized within 15 s. The combine effects of the catalysis reaction caused by the OH* radical and microwave heating led to an excellent microwave sterilization. Strongly absorptive microwave materials proved to be effective as microwave sterilization filters. Microwave sterilization is the most powerful method in the air purification system.

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