

# Measurement of excited singlet oxygen molecule in a vacuum sterilization system, using electric spin resonance (ESR) with a water-soluble polymer thin film

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## ABSTRACT

An advanced sterilization system employing active oxygen species (AOS) was investigated. We revealed in a previous paper that, by using active oxygen species generated from oxygen gas by ultraviolet irradiation in the chamber of the sterilization system, the survival curves of *Geobacillus stearothermophilus* spores ( $10^6$  CFU), a biological indicator (BI), showed exponential reduction, and that this sterilization was attributable to the UV-generated AOS. However, we had little knowledge of what specific types of AOS were primarily responsible for the sterilization effects. Therefore, in this study we investigated the principal sterilization contributors among the AOS generated by ultraviolet irradiation, using the electron spin resonance (ESR) technique, with 2,2,6,6-tetramethyl-4-piperidinol (TEMP) as a spin-label reagent for the excited state of atomic oxygen molecules ( $^1\text{O}_2$ ), and compared the respective sterilization effects of AOS and oxygen plasma system.

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## 1. Introduction

We have been investigating an advanced sterilization system employing active oxygen species (AOS), and have designed sterilization equipment which generates AOS from pure oxygen gas, using ultraviolet irradiation. The use of AOS has many advantages, such as low temperature ( $<60^\circ\text{C}$ ) and dry processing, in comparison with high-pressure steam sterilization conducted in autoclaves (AC:  $121^\circ\text{C}$  for 20 min); and no residual toxicant effects, in comparison with ethylene oxide gas (EOG) sterilization [1–3]. Moreover, in our system, the only raw materials required for sterilization are oxygen and a light source (ultraviolet radiation), thus the system is exceptionally safe and inexpensive.

Our former study reported that, in the chamber of the AOS sterilization apparatus, the survival curves of *Geobacillus stearothermophilus* spores ( $10^6$  CFU), the biological indicator (BI), showed exponential reduction, and that the sterilization was due to the AOS generated by ultraviolet irradiation [4–6]. However, we had little knowledge of the composition of the AOS generated in

the chamber, or of which AO species were principally responsible for the sterilization effects. In this sterilization system, which generates AOS from pure dry oxygen gas using ultraviolet irradiation, there are four atomic oxygen species candidates: excited singlet oxygen atom [ $\text{O}(^1\text{D})$ ], excited singlet oxygen molecules ( $^1\text{O}_2$ ), ground-state oxygen atom [ $\text{O}(^3\text{P})$ ] and ozone ( $\text{O}_3$ ) [7–10]. Of the four oxygen species,  $\text{O}_3$  has the lowest reactivity but the longest lifetime. It is likely that  $\text{O}_3$  acts on microorganism surfaces, and contributes to sterilization. However, we should not ignore the potential contribution of the most highly reactive oxygen molecules and atoms such as  $^1\text{O}_2$ ,  $\text{O}(^1\text{D})$  and  $\text{O}(^3\text{P})$ , even if their lifetime is extremely short [7]. Therefore, in this study we investigated the principal contributors to the sterilization effect in the present system, using the electron spin resonance (ESR) technique, with 2,2,6,6-tetramethyl-4-piperidinol (TEMP) as a spin-label reagent to specifically detect excited singlet oxygen production [11–15].

In addition, sterilization systems using plasma technology have been extensively studied in recent years [16–23]. We ourselves have previously investigated the inactivation of microorganisms resulting from exposure to atomic oxygen (ground-state oxygen atom) generated from a low pressure oxygen plasma (inductively coupled plasma source) [24,25]. Therefore, we here investigated the respective sterilization effects of an AOS sterilization system

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involving ultraviolet irradiation and a low pressure oxygen plasma system compared with the ozone sterilization system, and assessed the impact of AOS exposure.

In addition, we compared this AOS sterilization system to therapeutic modalities such as antimicrobial photodynamic therapy (APDT), which uses active oxygen species generated by methylene blue dye (MB) and 660 nm irradiation [26–28].

## 2. Experimental and methods

### 2.1. 1 sterilization system using active oxygen species, and operating procedure

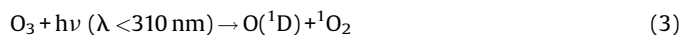
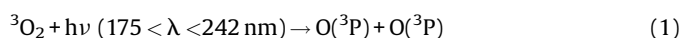
Fig. 1 shows a schematic diagram of the sterilization system that uses active oxygen species generated by ultraviolet irradiation of oxygen gas. The system consists of a chamber connected to a dry oxygen gas cylinder through a mass flow controller, as well as a vacuum pump, temperature controller, and power-supply unit. The effective capacity of the chamber is 45 l (W 400 × L 615 × H 400 mm), and two types of UV-lamp are installed in the chamber; one is a 200 W low pressure mercury lamp (UV-lamp-A: QGL200G-3, Iwasaki Electric), which emits ultraviolet radiation at both 185 and 253.7 nm, for generating active oxygen species, and the other is a 30 W low pressure mercury lamp (UV-lamp-B: QGL30-2, Iwasaki Electric), which emits ultraviolet radiation mainly at 253.7 nm, for resolving remaining ozone and accelerating the completion of the sterilization procedure. A mixing fan with heat sink is also installed, to stir the gases in the chamber. The oxygen cylinder contains industrial dry oxygen (greater than 99.9% purity), the mass flow controller is a MODEL 8550MC (KOFLOC), and the pump is a scroll vacuum pump (FO-0009A, Nippon Busch K.K.).

The operating procedure for the active oxygen species sterilization system is as follows. First, the vacuum pump is activated until a predetermined pressure level (generally 200 Pa) was achieved. Then the UV-lamp-A is activated and, after 1 min,

oxygen gas is infused at a rate of 50 l/min until a predetermined pressure level (generally 95 kPa) is achieved, followed by AOS treatment for a predetermined time. Subsequently, the UV-lamp-A is deactivated and the UV-lamp-B is activated for 5 min. The UV-lamp-B is then deactivated and the vacuum pump reactivated until the chamber is again evacuated. The chamber is then backfilled with air, concluding the procedure [4–6].

### 2.2. Active oxygen species generated by ultraviolet irradiation

The mechanisms involved in the photochemical reaction that generates AOS through ultraviolet irradiation may be explained as follows [7–9]. In oxygen atmospheric conditions, under ultraviolet irradiation ( $175 < \lambda < 242$  nm), the ground-state oxygen molecules ( $^3\text{O}_2$ ) are broken down into two ground-state oxygen atoms [ $\text{O}(^3\text{P})$ ] [Eq. (1)], each of which immediately combines with  $^3\text{O}_2$  to produce ozone ( $\text{O}_3$ ) [Eq. (2)]. Then, through further ultraviolet irradiation ( $\lambda < 310$  nm), the  $\text{O}_3$  is broken down to produce excited oxygen molecules ( $^1\text{O}_2$ ) and excited oxygen atoms [ $\text{O}(^1\text{D})$ ] [Eq. (3)]:



where  $^3\text{O}_2$  is a stable ground-state triplet oxygen molecule,  $\text{O}(^3\text{P})$  is a ground-state triplet oxygen atom,  $\text{O}_3$  is ozone,  $^1\text{O}_2$  is an excited singlet oxygen molecule, and  $\text{O}(^1\text{D})$  is an excited singlet oxygen atom. Under dry, oxygen-rich conditions in the chamber, when UV lamp A (which emits ultraviolet radiation at both 185 and 254 nm) is activated,  $\text{O}(^3\text{P})$  and  $\text{O}_3$  are generated by the 185 nm irradiation, and  $\text{O}(^1\text{D})$  and  $^1\text{O}_2$  are generated by further irradiation at 254 nm. Thus, when using UV-lamp-A, we may obtain AOS such as  $\text{O}(^3\text{P})$ ,  $\text{O}(^1\text{D})$ , and  $^1\text{O}_2$ .

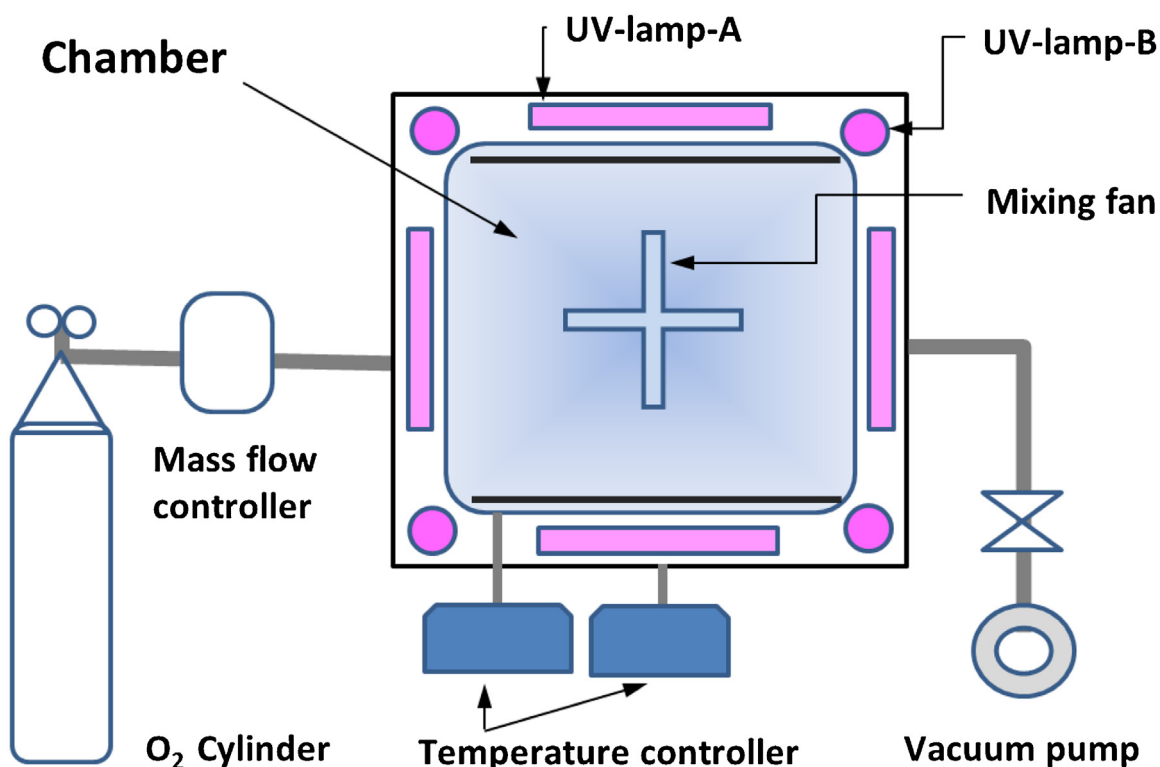


Fig. 1. Schematic diagram of the sterilization system using active oxygen species (AOS sterilization equipment).

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