A Colorimetric Indicator for Detection of Hydroxyl Radicals in Atmosphere Using a Methylene Blue Dye Based on Nafion Film

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Abstract

Methylene blue based on the nafion film was prepared and chemical reactions in contact with active oxygen species were estimated. In addition, decolorization mechanism of the film due to exposure of the active oxygen species is discussed. The active oxygen species was exposed to the methylene blue based on the nafion film in low and high humidity conditions. Although decolorization of the film was hardly observed when it was exposed in the ozone and the active oxygen species in low humidity condition, the decolorization was observed when it was exposed in the high humidity condition. It is considered that the decolorization of the film due the active oxygen species exposure relates to the degradation reaction of the methylene blue molecule due to hydroxyl radicals.

Keywords: methylene blue, nafion, active oxygen, hydroxyl radical, degradation, indicator

1. Introduction

Active oxygen species (AOS) generated under atmosphere or low vacuum condition can be applied for various industrial processes owing to extremely strong oxidative abilities. It is well known that excited singlet oxygen atom [$O(^1D)$], ozone (O_3) and hydroxyl radicals (OH^*), are of interest and value in surface modification, cleaning and oxidation processes. Chemical kinetics of the generation of these AOS had been already reported [1-4]. We have already reported that the AOS generated under ultraviolet (UV) lamps, whose wavelength are 185 and 254 nm, or oxygen plasma can be used for a sterilization process of microorganisms [5]. We have also reported that an advanced sterilization system employing the AOS has been investigated. New sterilization equipment installed an evacuation system, which generates AOS from pure oxygen gas under ultraviolet irradiation, was designed [5].

We examined the critical conditions for sterilization using the quartz crystal microbalance (QCM) method that provides a physical indicator for sterility evaluation. We had already reported on the AOS monitoring using a QCM with silver thin film as the detection layers, however, this QCM system has a serious drawback that the sensing values become saturated during the

measurement owing to oxidized deterioration of the silver surface [6, 7]. Fluorocarbon thin film deposited by r.f.sputtering with a poly(tetrafluoroethylene) (PTFE) target on the quartz crystal was investigated to determine the suitability for application in the AOS sensors. The AOS generated under ultraviolet irradiation was successfully detected from frequency shifts using the QCM electrode coated with the fluorocarbon thin films [8]. We succeed in quantification of the $O(^1D)$ and OH^* in atmosphere due to the electron spin resonance (ESR) measurements [9]. However, it is difficult to evaluate effect of each AOS on the sterilization of microorganisms using the QCM system.

Methylene blue (MB) is one of redox dyes and used for colourimetric oxygen indicator [10, 11]. The MB was used for the investigation of the photochemical stability of biopolymers [12]. Among various dyes, MB is known difficult to be degraded by conventional waste water treatment methods due to its stability [13]. On the other hand, it is well known that ozone decolorizes the MB solution [14]. As excited state of atomic oxygen has higher oxidation ability compared to that of the ozone and the decolorization reaction occurs rapidly, it is difficult to use in the high concentration environment of the excited state of atomic oxygen [7]. Hydroxyl radical has the highest oxidation ability in the AOS, and it is important to enhance the MB durability for the oxidation. In addition, it has been required to form a solid film containing the MB dye when it is used as an indicator of the new sterilization process employing an evacuation system for microorganisms.

Nafion is one of fluorocarbon polymers which contains sulfonate group.

Hassan et al. reported on an electrochemical sensor using MB dye based on nafion as an indicator [15]. Seger et al. reported on monitoring system of proton activity in a proton-exchange membrane (nafion) introduced by incorporating the MB as an indicator dye [16]. In this paper, we reveal the AOS indicator using the MB based on the nafion film. This film was installed into a sterilization bag to eliminate effects of the UV lights on the decolorization reaction. We have already reported that the UV lights cannot penetrate the sterilization bag, but these AOS can permeate it [5]. This indicator indicates a selective decolorization reaction in high humidity condition, which suggests that hydroxyl radicals relate to the decolorization reaction of the MB dye based on nafion film.

2. Materials and methods

2.1 Preparation of methylene blue dye based on nafion film

Methylene blue (98.5%) and nafion® (DE521, 5% nafion® dispersion solution) were purchased from Wako Pure Chemical Industries, Ltd. The MB, whose concentration was 0.6 g/L, was dissolved in distilled water. 2mL of the MB aqueous solution was mixed with 1mL of the nafion aqueous solution, which is 5.0 % dispersion solution, and stirred at room temperature. The MB dye based on the nafion film was prepared by the following; 0.3 mL of mixture is dispersed onto the glass slide substrate (76 mm × 26 mm), and dried at 37 °C for 10 min. The film thickness was estimated to be $5 \pm 1 \mu\text{m}$ by measuring height between the glass substrate and coating film using a profilometer, Dektak XT (Bruker Co., Ltd.).

2.2 AOS exposure

AOS exposure was performed according to our previous paper [9]. The AOS system (Active-Dry® Iwasaki Electric Co., Ltd. Japan) equipped with three low-pressure mercury UV lamps was used for the generation of active oxygen species. The combined power of the lamps was 16 W, comprising of two 6 W lamps and one 4 W lamp. The AOS were generated in low and high humidity conditions. For low humidity condition (<20%RH), prior to being introduced into the AOS system, dry air was passed through a bottle with silica gel which was kept at 30 °C. For high humidity condition (>90%RH), air was introduced through a water bottle which was also kept at 30 °C by introducing warm air continuously. The air pumping was stopped when the active oxygen exposure was performed. The wavelengths of these UV lights were 185 and 254 nm, respectively. The excited state of oxygen is mainly produced in a dry condition, and hydroxyl radical mainly produced in a humid condition [9]. The temperature in the system was also kept at 30 °C by introducing warm air continuously. Ozone can be easily produced by an ozonizer (REI-SEA, YGR-203N). The ozone generated by the ozonizer was supplied into the AOS system without irradiation of UV light. All of the MB dyes based on the nafion films were placed into a sterilization bag (Johnson & Johnson K.K.) to prevent decomposition of the MB and nafion due to the UV irradiation. The sterilization bag is composed of plastic film and nonwoven. The UV–Visible (vis) light within a wavelength between 190 and 800 nm cannot penetrate the nonwoven (under the detection limit; <0.03 %).

Although visible light can permeate into the plastic film side, the UV light, whose wavelengths are 185 and 254 nm, can hardly permeate the plastic film. In this study, the AOS and UV lights were irradiated at the nonwoven side.

2.3 Evaluation of the decolorization

Decolorization of the MB dye based on the nafion film due to the AOS exposure was first evaluated using a UV–vis spectrophotometer (Shimadzu UV-2450) within a wavelength between 190 and 800 nm. For the second, the MB dye based on the nafion film was dissolved into ethanol using an ultrasonication, and measured transmittance of the solution within the same wavelength regions using the same UV–vis spectrophotometer.

2.4 Analyses of molecular structures of the MB dye based on the nafion film

Molecular structures of the MB dye based on the nafion film were estimated with fourier transform infrared (FT-IR) spectrometry (FT-720, Horiba, Co. Ltd.) using the KBr tablet method, and proton NMR (^1H NMR). Proton NMR (^1H NMR) spectra were performed on a Bruker 500 Hz Ultrashield spectrometer using D_2O as a solvent for dissolving the MB dye based on the nafion film.

3. Results and discussion

3-1. Hydroxyl radical (OH^*) indicator characteristics using the MB dye based on the nafion film

Figure 1 illustrates decolorization of the MB dye based on the nafion

film deposited onto glass substrate after the AOS exposure for 30 min. The MB dye based on the nafion film was decolorized and turned to transparent film by the AOS exposure in the high humidity condition, although it was hardly decolorized by the AOS exposure in the low humidity condition. It is well known that the AOS generated under the UV lights in high humidity condition is hydroperoxide (HO_2) as well as the OH^* , however the OH^* is of special interest from the point of view of stability and oxidation ability in atmosphere [9]. The generated superoxide anion and OH^* are the major reactive species produced during photocatalytic reactions [17]. In addition, generation amount of the OH^* depends on the humidity condition [5], and it is considered that decolorization of the film in the high humidity condition is due to the OH^* . Fig. 2 shows visible light transmittance spectra of the MB dye based on the nafion films before and after the AOS exposure for 30 min in low and high humidity conditions. The transmittance spectrum of the film was hardly changed even after the AOS exposure in the low humidity condition, however the transmittance around the 750 nm was dramatically increased compared to those of the film before and after the AOS exposure in the low humidity condition. Mika et al. already reported that the MB was strongly sorbed to the nafion, and distinct band at about 750 nm , which attributed to the presence of protonated methylene blue, appeared [18]. In this experiment, this distinct band can also be observed, and it decreased due to the AOS exposure in the high humidity condition, which suggests that the protonated methylene blue is decomposed by the OH^* .

The MB dye based on the nafion film has a serious drawback in

uniformity of the film as shown in Fig. 1. To evaluate sensing characteristics quantitatively, the film after the AOS exposure was dissolved into ethanol (99.5%) and the transmittance spectrum of the solutions was measured. In addition to these humidity conditions, ozone generated by the ozonizer, whose concentration was 140 ppm, was also used for the decolorization experiment. The ozone exposure was kept for 30 min in the dry condition. Fig. 3 shows decolorization of the films dissolved into the ethanol solutions. The ozone exposure hardly affects the decolorization, and the AOS exposure in the low humidity condition slightly affects the decolorization. On the contrary, the AOS exposure in the high humidity condition dramatically affects these decolorization. Fig. 4 indicates the transmittance spectra of the film solutions after the AOS exposure. The transmittance spectrum after the AOS exposure for 30 min in low the humidity condition was also closed to the spectrum before the exposure, however the spectrum after the exposure in the high humidity condition was different, i.e., distinct bands at 610 and 660 nm were dramatically decreased. These two bands attributed to characteristic absorbance peaks of the MB dimer and MB monomer, respectively [19]. These results suggest that the MB monomer and dimer coexist in the ethanol solution, and these molecules are decomposed by the AOS exposure in the high humidity condition.

Fig. 5 indicates relationship between peak absorbance at 660 nm and exposure time of the AOS in the high humidity condition. The peak absorbance, A , can be calculated by the transmittance, T (%), at 660 nm, according to Lambert–Beer Law [20].

$$A = 2 - \log_{10} T \text{ ----- (1)}$$

Concentration of the MB in the MB dye based on the nafion film is calculated to be 24 ppm, and about 75 % of the MB was decomposed after the 30 min irradiation.

The absorbance was almost linearly decreased with exposure time of the AOS in the high humidity condition. It is well known that OH* and O(¹D) are generated under UV light at 365 nm [21] and UV – vis light at ~1270 nm in a water, respectively [22]. However, it is considered that these AOS due to the photoexcitation reactions cannot be generated in the nonwoven bag, as these UV and visible lights hardly penetrate the nonwoven. These results suggest that the MB dye based on the nafion film is a potential material for the detection of the OH* in the nonwoven bag.

3-2. Decolorization mechanism of the MB dye based on the nafion film

Nafion is one of anionic polymers, and methylene blue cations (MB⁺) are considered to form ionic bond with the nafion. We estimated chemical bonding site of the MB and nafion using the ¹H NMR. Fig. 6 shows ¹H NMR spectra of MB dye and the MB dye based on the nafion film dissolved into D₂O. Several characteristic peaks of the MB can be observed around 3.1, 6.8, 7.0 and 7.3 ppm, which are assigned to the protons in functional groups as shown in Fig. 7(a). Chemical shifts can be observed at each characteristic peak due to the mixture of nafion, which suggests that fluorine in nafion

molecule affects these chemical shifts in the MB molecule, because S-O-S bond forms between thiazine in the MB and sulfonate group in the nafion (Fig.7(a)(b)).

After the AOS exposure to the MB for 30 min in the high humidity condition, it was analyzed with the ^1H NMR. Fig. 8 indicates the ^1H NMR spectra of the MB dye before and after the AOS exposure. A peak at 3.2 ppm, which was assigned to $\text{CH}_3\text{-N}$, disappeared due to the AOS exposure. This result indicates that the MB is decomposed by the OH^* . Fig. 9 indicates the ^1H NMR spectra of the MB based on the nafion film before and after the AOS exposure. The peak at 3.2 ppm disappeared due to the AOS exposure as well as in the case of the MB dye. In addition, three small peaks between 6.5 and 7.5 ppm correspond to the hydrogen in the benzene ring. These three peaks also disappeared due to the AOS exposure. Generally, the OH^* reacts with benzene ring. It is well known that benzene ring is cleaved by AOS reactions and decomposed due to quantum chemical calculations [23-25]. These results suggest that the $\text{CH}_3\text{-N}$ group in the MB had disappeared due to the AOS exposure accompanied by the decomposition of the benzene ring.

From these results, it is considered that the decolorization mechanism of the MB dye based on the nafion film is mainly caused by the cleavage of the benzene ring and decomposition of the $\text{CH}_3\text{-N}$ group in the MB molecule.

Fig. 10 shows FT-IR spectra of the MB dye based on the nafion films before and after the AOS exposure for 30 min in low and high humidity conditions. Although peaks between 1100 and 1300 cm^{-1} still remain in a spectrum of the film after the AOS exposure in the low humidity condition,

those cannot be observed in the spectrum after the AOS exposure in the high humidity condition. It is already known that the $\text{CH}_3\text{-N}$ group in the MB molecule can be observed at 1240 and 1182 cm^{-1} [26]. In addition, atomic ratios of nitrogen for carbon in the MB dye based on the nafion films before and after the AOS exposure for 30 min in low and high humidity conditions are 0.03, 0.02 and 0.01, respectively from the results of x-ray photoelectron spectroscopy analyses. These results suggest that the decolorization mechanism of the MB dye based on the nafion film relates to the degradation of the $\text{CH}_3\text{-N}$ group and benzene ring in the MB molecule. The MB degradation reaction due to the OH^* is already reported [27-29]. As mentioned previously, the oxidation abilities of $\text{O}(^1\text{D})$ and O_3 are lower than that of the OH^* , and it is considered that $\text{O}(^1\text{D})$ and O_3 can hardly oxidize the MB dye and decompose the $\text{CH}_3\text{-N}$ group and benzene ring in the MB molecule when the MB dye based on nafion film was used for the AOS reaction.

4. Conclusion

The AOS indicator characteristics were evaluated using the MB dye based on the nafion film to develop an indicator of sterilization process for microorganisms.

The MB dye and nafion combine with S-O-S bond between thiazine in the MB and sulfonate group in the nafion, which affects the chemical shifts of protons in the MB molecule.

The MB dye based on the nafion film showed specific decolorization

reaction with the AOS generated in the high humidity condition, and the O(¹D) and ozone in the AOS hardly react with the MB. The results of ¹H-NMR and FT-IR analyses suggest that the OH* in the AOS decomposes CH₃-N group and benzene ring in the MB.

We conclude that the MB dye based on the nafion film is a potential material to be used in a colorimetric AOS indicator to detect the OH*.

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Figure captions

Fig.1 Decolorization of the MB dye based on the nafion film deposited onto glass substrate due to the AOS exposure for 30 min in the low and high humidity conditions.

Fig.2 Visible light transmittance spectra of the MB dye based on the nafion films before and after the AOS exposure for 30 min in low and high humidity conditions.

Fig.3 Decolorization of the films dissolved into the ethanol solutions: (a) before dissolution of the film, (b) the AOS exposure in the low humidity condition, (c) the AOS exposure in the high humidity condition and (d) ozone exposure for 30 min, respectively.

Fig.4 Transmittance spectra of the extracted MB dye based on the nafion film solutions before and after the AOS exposure in the low and high humidity conditions.

Fig.5 Relationship between the MB dye concentration calculated from peak absorbance at 660 nm and exposure time of the AOS in the high humidity condition.

Fig.6 ¹H NMR spectra of MB dye and the MB dye based on the nafion film

dissolved into D₂O.

Fig.7 Molecular structures of the MB and nafion: (a) MB and chemical shifts of ¹H-NMR, and (b) nafion.

Fig.8 ¹H NMR spectra of the MB dye before and after the AOS exposure in the high humidity condition.

Fig.9 ¹H NMR spectra of the MB based on the nafion film before and after the AOS exposure in the high humidity condition.

Fig.10 FT-IR spectra of the MB dye based on the nafion films before and after the AOS exposure for 30 min in low and high humidity conditions.

Fig.1

Without treatment

High humidity condition

Low humidity condition

Fig.2

Fig.3

Fig.4

Fig.5

Fig.6

Fig.7

(a)

(b)

Fig.8

Fig.9

Fig. 10