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Electron-spin-resonance analysis of liquid irradiated with reactive oxygen radicals for inactivating microorganisms

微生物殺菌のための酸素ラジカル照射された溶液の電子スピン共鳴解析

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Using the atmospheric-oxygen radical-source, which can treat samples with only neutral oxygen radicals, we elucidated quantitatively that inactivation rates of *Esherichia. coli* in liquid phase were independent of the pH values in the solutions. In this study, we have analyzed activated species, such as OH radical and hydrogen peroxide generated in the solutions with different pH values treated with neutral oxygen radicals, by using electron spin resonance method and chemical probe method.

1, Introduction

Recently, various applications in decomposition of organic matter, and inacticatvation of microorganisms using gas-liquid plasmas have been intensively studied. Plasma provides various factors such as UV radiation, reactive oxygen species (ROS), ions, electrons, and ozone, simultaneously. Many papers reported that ROS were effective to inactivate microorganism [1],[2]. Ikawa *et al*. reported that *E. coli* in solutions was higher inactivation while low pH conditions. The inactivation of *E. coli* using a low-frequency He plasma jet was greatly contributed by ROS, particular for superoxide anion radicals (charged particles) in liquids[3]. However, effects of the individual activated species in gas phase on inactivation have not been understood quantitatively and also not controlled the interaction of biological samples and plasmas. Therefore we focused on effects of neutral oxygen radicals.

We inactivated the *Esherichia coli* in solution, treated by using an atmospheric pressure oxygen radical source supplying only neutral oxygen radicals. Previously the inactivation rates of *E. coli* in liquid treated with neutral oxygen radicals were successfully evaluated quantitatively, and the results of inactivation rates were not depended on the pH condition of neutral or acidic solutions [4]. Therefore, it is required to clarify the inactivation mechanism including the interactions between oxygen radicals and liquids.

In this report, we prepared both acid and neutral solutions by treatments with neutral oxygen radicals. Then we analyzed chemical reactive species generated in the liquid using methods; electron spin resonance (ESR) and chemical probe, and we will discuss the inactivation mechanism in liquids treated by neutral oxygen radicals.

2, Experimental procedure

Figure 1 illustrates experimental procedure for sample preparation. An atmospheric-pressure oxygen-radical source (Fuji Machine MFG. Co., Ltd.; tough plasma) can supply only neutral oxygen radicals with inert carrier gas, without ultraviolet rays and charged particles (an electrons and ions). Operating conditions were Ar/O_2 plasma at atmospheric pressure.

Buffered solutions were prepared by adding chemicals into MillQ water. Solutions adjusted to pH 3.7 (sodium citrate buffer) and pH 6.8 (pure water).

For detection of hydroxyl radical, we employed spin-trapping method. Before plasma treatments, DMPO (5,5- Dymetyl-1- pyrroline–N-oxide) trap agent was added to be 1% of the solution of 500μl.

8wellchamber filled samples were located on the stage below the radical head. The stage was enclosed with a plastic cover in order to avoiding any influences of ambient air. 0.6% O_2 added Ar gas with total gas flow rate of 5 L/min was supplied to the radical head. A distance from the radical-exit slit of the radical head to the liquid surface was adjusted 10 mm. Treatment time was typically for 3 min. Just after treatments within a minute, ESR measurements were carried out.

Quantities of hydrogen peroxide generated in the solutions by neutral oxygen radical treatment were measured using the chemical probe method using Amplex Red.

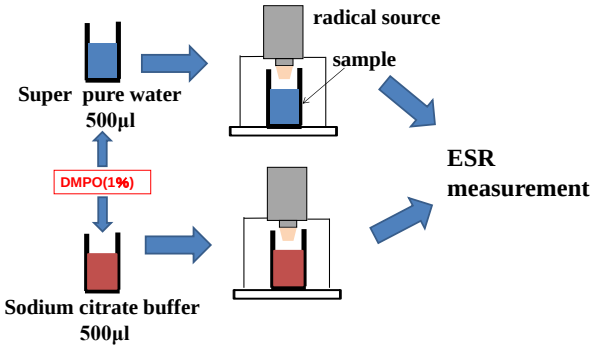

Fig. 1 Experimental procedure.

3. Results and discussion

Figures 2 and 3 show the ESR signal of ultrapure water and sodium citrate buffer treated with neutral oxygen radicals for 3 min. The signals of DMPO-OH were detected in both the acidic and neutral solutions. The results indicate that OH radicals generate in the both solutions treated with neutral oxygen radicals.

Figure 4 shows hydrogen peroxide concentration generated in the solutions as a function of neutral-oxygen radical-treatment time. The amount of hydrogen peroxide increased linearly with increasing treatment time. At the treatment time of 10 min, approximately ~50 µM hydrogen peroxide were detected in the sodium citrate buffer as well as in the ultrapure water. According to the previous report [5], same inactivation rates were necessary obtained at the condition of much amounts of hydrogen peroxide. Namely, high inactivation rates obtained with less hydrogen peroxide concentration. This possibly implies that the oxygen radical treatments generate a certain unspecified chemical reactive species in the solutions. Further investigation needs for future topic.

4. Summary

We have investigated the activated species generated in liquids treated with neutral oxygen radicals for elucidating inactivation mechanism using ESR and a chemical probe. The results showed the generations of OH radical and hydrogen peroxide in the acid and the neutral solutions treated with neutral oxygen radical and suggest that the other active species except H_2O_2 mainly contribute to the inactivation.

Acknowledgements

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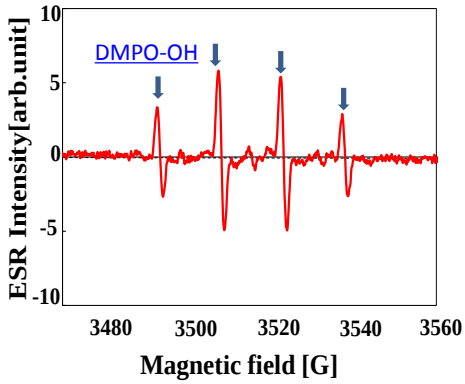

Fig. 2. ESR signal of super pure water treated with oxygen radicals.

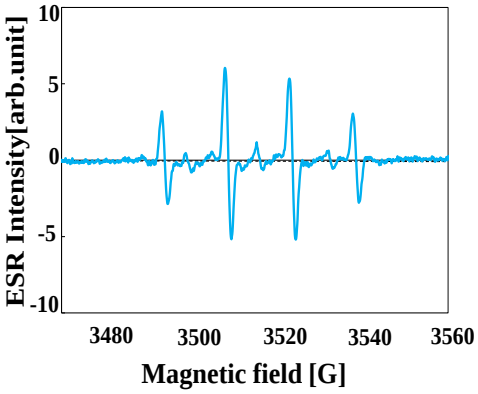

Fig. 3. ESR signal of sodium citrate buffer treated with oxygen radicals.

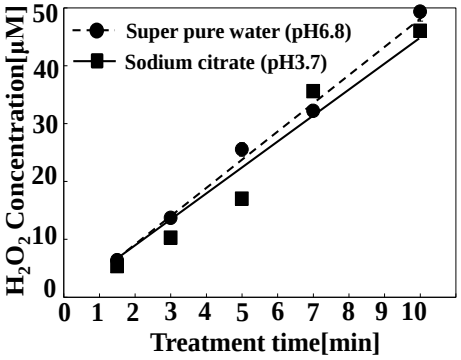

Fig. 4. Amount of hydrogen peroxide generated in the acid neutral solutions treated by neutral oxygen radicals as a function of treatment time.