2nd Asia-Pacific Conference on Plasma Physics, 12-17,11.2018, Kanazawa, Japan

Long-term bactericidal effect and reactive oxygen and nitrogen species (RONS) chemistry of radical-activated water

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Bactericidal effect of plasma-activated water (PAW) is well known since Ikawa and his colleagues introduced. [1,2] In their reports, the limited shelf lifetime about 8 min. and strong dependency of bactericidal effect on pH value were revealed. On the other hand, Traylor and his colleagues reported long-term antibacterial efficacy of PAW which showed 2.4-log reduction with a week aged PAW. [3] The both previous studies showed a strong correlation between the bactericidal effect and plasma generated reactive oxygen and nitrogen species (RONS).

Recently, we developed non-equilibrium atmospheric-pressure radical sources of which enables to generate either atomic oxygen or nitric oxide (NO). The radical density can be varied as change of feed gas flow rate. In this study, we used NO radicals to activate water namely radical-activated water (RAW). The both long-term evaluation of bactericidal effect using colony forming unit (CFU) of *Escherichia coli* (*E. coli*) and RONS and O_2 chemistry using a conventional UV-vis spectrophotometry, respectively, for a one-month period.

In the experiment, distilled deionized water (DDW) was irradiated with NO radicals using a commercially available radical source (Fuji Machine, Tough Plasma), which can only supply neutral oxygen and nitrogen radicals without charged species and high energetic UV photons. A large volume of DDW (700 mL) was irradiated with NO radicals for 10 min. The RAW sample was dispensed into several tens glass bottles by 20 mL and stored until the investigation. We used UV-vis spectrophotometry and deconvolution analysis to measure the RONS concentrations in RAW. Subsequently, *E. coli* (10^7/mL) was suspended into RAW and this was cultured for 24 h at 30 ^oC, then CFU was investigated.

Here we show that the very strong bactericidal effect of RAW and RONS chemistry regarding H_2O_2, NO_2^- and NO_3^- concentrations in the liquid generated by NO radical irradiation. The absorption profiles and chemical compositions are very similar to the case of PAW. As the result of time evolution measurement, H_2O_2 concentration was quite stable during the period and *E. coli* was sterilized using even 7-day-stored RAW samples. Interestingly, *E. coli* colony was counted after two weeks up to a month for our observation period.

This work was supported by the MEXT-Supported Program for the Strategic Research Foundation at Private Universities (S1511021) and a JSPS KAKENHI Grant Number JP17K05099. We acknowledge a project for Promoting Research Center in Meijo University.

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