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## Long-term Investigation of Radical-activated Water

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Bactericidal effect of plasma-activated water (PAW) is well known since Ikawa and his colleagues introduced. Limited shelf lifetime and strong dependency of pH value were revealed in the paper. 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. 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 RONS chemistry using UV-vis spectrophotometry and bactericidal effect using colony forming unit (CFU) of *Escherichia coli* (*E. coli*) 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 °C, then CFU was investigated.

Here we show that  $\text{H}_2\text{O}_2$ ,  $\text{NO}_2^-$  and  $\text{NO}_3^-$  in DDW were 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,  $\text{H}_2\text{O}_2$  concentration was quite stable during the period and *E. coli* was sterilized using even 7-day-stored samples. Interestingly, *E. coli* colony was counted after two weeks up to a month for our observation period.

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