7th Asia-Pacific Conference on Plasma Physics, 12-17 Nov, 2023 at Port Messe Nagoya

The importance of cold plasma-generated short-lived reactive species, especially superoxide anion radicals in antibiofilm activities

Binbin Xia[1], Heema Kumari Nilesh Vyas[1], Renwu Zhou[2], Tianqi Zhang[1], Jungmi Hong[1], Joanna G. Rothwell[3], Scott A. Rice[4], Dee Carter[3], Kostya (Ken) Ostrikov[5], Patrick J. Cullen[1] and Anne Mai-Prochnow[1]

[1] School of Chemical and Biomolecular Engineering, The University of Sydney, [2] State Key Laboratory of Electrical Insulation and Power Equipment, Center for Plasma Biomedicine, Xi'an Jiaotong University, [3] School of Life and Environmental Sciences, The University of Sydney, [4] Agriculture and Food, Microbiomes for One Systems Health, Commonwealth Scientific and Industrial Research Organisation, [5] School of Chemistry and Physics and QUT Centre for Biomedical Technologies, Queensland University of Technology

e-mail (speaker): anne.mai-prochnow@sydney.edu.au

Introduction: Microbial biofilms are responsible for the contamination of food, filters, membranes, and equipment as well as causing diseases. Thus, these communities of microorganisms that live attached to a surface are a risk to human health and cost millions of dollars in equipment repair and cleaning. A new reliable decontamination method that does not cause resistance is needed. Cold plasma or plasma-activated water (PAW) exhibits powerful disinfectant activity. However, the optimal generating conditions, such as the choice of gas used to produce PAW, remain unclear.

Methods: Our study utilizes PAW generated with a bubble spark discharge (BSD) reactor connected to different gas sources (argon, nitrogen, oxygen, and atmospheric air). Biofilms of *Escherichia coli* that were grown on stainless-steel surfaces were treated in situ to optimize the biofilm removal efficacy. Antimicrobial activity was measured using colony forming units (CFU) counts, microscopic imaging, and intracellular ROS measurements. The PAW was characterized by OES, scavenger assays and electron paramagnetic resonance.

Results: We demonstrate that PAW generated using oxygen (PAW-O_2) is the most effective and can completely remove *E. coli* biofilms on stainless steel surfaces (Fig. 1).

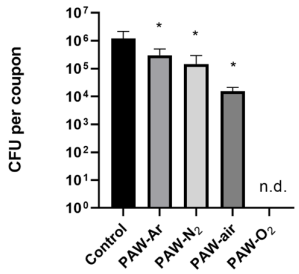

Fig. 1: Reduction of *E. coli* biofilms using PAW generated in argon, nitrogen, air, and oxygen assessed by CFU

OES measurements support the experimental observations on the generation of reactive species such as OH^*, O_2^-, NO^*, and excited atoms in the gas phase of BSD plasma, which then leads to the solvation and diffusion of RONS at the bubble-water interface and formation of activated water. In the oxygen BSD plasma, various forms of reactive oxygen species can be generated including excited oxygen molecular and atomic species, ions and ozone, superoxide anion radicals and hydrogen radicals through the interaction with water or its derivatives such as OH^- or HO_2 [1]. Moreover, for the first time, we show that a short-lived reactive oxygen species, the superoxide anion radical (•O2-), plays an important role in the inactivation of *E. coli* biofilms. Using scavengers and electron paramagnetic resonance spectra the superoxide anion radical is detected and shown to cause microbial biofilm killing (Fig. 2)

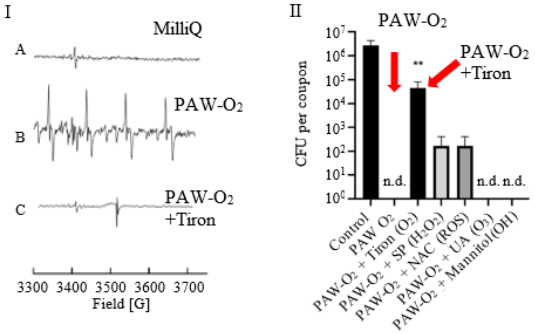

Fig. 2: (I) Superoxide detection using electron paramagnetic resonance spectra A MQ water control; B. PAW-O_2 C. PAW-O_2 +Tiron. (II) The addition of the superoxide scavenger Tiron prevents bacterial killing of PAW

Conclusion: The results of our study show that PAW generating input gas is important for RONS generation. This has implications for biofilm removal strategies, including drinking and wastewater pipes, filter, and membrane cleaning, but also equipment decontamination and sterilization of food products or medical devices where conventional disinfection is not suitable [2].

References

[1] Bolouki, et al. (2021) Applied Sciences 11(13)
[2] Xia, et al. (2023) Journal of Environmental Chemical Engineering