Efficacy of plasma-generated ozone in bioburden decontamination

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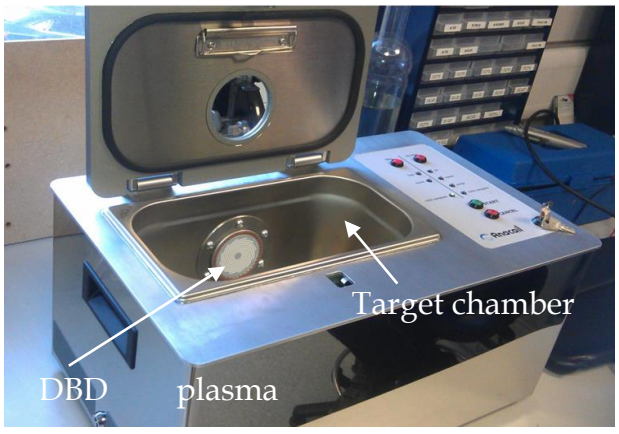
We show recent results of the efficacy of ozone, generated by cold plasma DBD discharge system, in the reduction of bioburden in various practical contexts. The patented plasma system is designed to generate ozone *in situ*, without endangering the operator, using the ambient air; in one configuration, the system can generate significant ozone concentrations in sealed packages from the outside, without compromising the seal. We demonstrate the performance of this system in a variety of contexts, with particular relevance to high level decontamination of medical devices, and also possible applications in disinfecting plumbing components. Only the plasma effluent impinges on the target: the plasma does not make contact. Our experiments show effective biocidal, virucidal, mycobactericidal and fungicidal treatments are possible, both *in* vitro and in realistic conditions.

1. Introduction

Cold plasma generation of ozone has a long history, primarily in water treatment plants as an alternative to chlorination [1]. Ozone is a very strong oxidising agent and consequently is an effective biocide. However, it is a difficult chemical to handle, because not only is it extremely hazardous to humans, it has a short half-life (a matter of hours at room temperature) and so must be generated on demand.

2.1 Plasma device

The novelty in our approach lies in the design of a plasma system that allows ozone to be generated inside sealed containers, but by an electrode system imposed from the outside [2]. In this system, there is no requirement to open the package for access, nor is any feedstock gas required. The electrode system attaches to the flexible package surface by suction, effectively making the package material an extra dielectric layer. Electric fields are expressed from the electrodes through the package to strike a low-energy plasma in the interior, which then generates ozone. The circulation of that ozone around the



package interior ensures any target contained within is exposed to a powerful biocide. Typically, the ozone levels can reach in excess of 1000ppm in 20s, for 1 litre packages. The system used is shown in Figure 1: treatment targets are packaged and placed inside the chamber, attaching to the electrode plate via vacuum suction. Treatment cycles are generally 100s, with the target left for 1 hour before retrieval and testing: this allows the ozone to decay naturally, and prolongs the exposure of the bioburden to the ozone. This separation of plasma treatment time and the target dwell time is very practical: multiple, separate targets can be sequentially treated with a single plasma source, and the targets remain safely packaged whilst decontamination proceeds.

2.2. Results

In vitro testing under dirty conditions (3.0g/l bovine albumin + 3.0ml/l sheep erythrocytes: Efficacy as a biocidal, virucidal, mycobatericidal and fungicidal device was demonstrated by testing, via an independent accredited microbiological laboratory (BluTest [3]), petri dishes containing *Salmonella enteritidis* NCTC 13346, *Listeria monocytogenes* NCTC 7973, *Escherichia coli O157* NCTC 12900, *Clostridium difficile* NCTC 11209, Murine norovirus s99/RAW 264.7 cells, *Mycobacterium terrae* ATCC 15755, *Aspergillus brasiliensis ATCC 16404*. In each case, tests were done under dirty conditions and produced at least a 4 $\log_{10}$ reduction in viable organisms. Treatment of inoculated surrogate lumens (2mm, 1.5m) showed > 6 $\log_{10}$ reduction in *P. aeruginosa*, and aseptic storage exceeding 6 weeks.

3. References

[1] Gerrity D, Snyder S: *Ozone-Sci Eng* 2011, **33**(4):253-266.

[2] Patent: Plasma generation and use of plasma generation apparatus WO 2011055113 A1

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