FINAL REPORT

Antimicrobial Performance of Ozone System

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1. Introduction

Aqualogic NT Ltd produces an ozone device for generating ozone water for a range of applications. The University of Dundee will collaborate with the company to evaluate the anti-microbial efficiency of ozone water from the device. As ozone has strong antimicrobial properties, ozoned water can be used as a surface sanitizer in food preparation, cooking and serving areas and general kitchen surfaces. Furthermore, ozone is at least 100x stronger and reacts 3100x faster than chlorine as a disinfectant. Chlorine reacts with organisms (bacteria, meat) forming highly toxic carcinogen compounds called THMs (tri-halomethanes). By comparison, ozone leaves no trace of residual products upon oxidative reaction. The use of ozone water will reduce chemicals needed by 50% and food spoilage by up to 50%. The results will help to improve their ozone generator efficiency and their products' competitiveness.

Biofilms occur in a wide variety of systems, such as in food preparation, cooking and serving areas and general kitchen surfaces. The bacteria in a biofilm are more resistant to antimicrobial agents as microbes excrete polymeric exopolysacharrides (EPS) film to protect them from the conventional antimicrobial reagents. The objective of this project is to evaluate the efficacy of ozonated water against biofilm formation originated from both gram-positive bacteria and gram-negative bacteria. Supported by Innovation Voucher Scheme Ay 2013-14, Scottish Funding Council, the anti-bacterial efficiency of Aqualogic NT Ltd' s ozone device has been evaluated.

2. Materials and methods

2.1 Types of bacteria

In this study, the antimicrobial assays of ozonated water collected from the ozone device (Aqualogic NT Limited) were performed at the Biological and Nanomaterials Lab, University of Dundee. The antimicrobial effects of ozonated water were evaluated against both gram-positive bacteria (*Staphylococcus aureus* (F1557)) and gram-negative bacteria (*Escherichia coli WT* (F1693), *Pseudomonas aeruginosa* (ATCC 33347)), which were obtained from Institute of Infection and Immunity, Nottingham University, UK. The strains were subcultured and preserved in 15% glycerol in TSB (Tryptone Soya Broth, Oxoid®, UK) as frozen stock at −80 °C. For all microbial tests, TSA (Tryptone Soya Agar) plates were streaked out with a loop from the frozen stock and grown overnight at 37 °C. A single colony was inoculated in 10 ml TSB and grown statically overnight at 37 °C. 500 ml from this culture were further inoculated into 100 ml TSB in a conical flask and grown in a shaker-incubator at 37 °C and 250 rpm. The culture was grown to mid-exponential phase. The strains were harvested by centrifuging at 4500 rpm for 5 min at −4 °C, washed once in sterile distilled water and re-suspended in PBS (Phosphate Buffered Saline) at a 10^6 CFU/ml concentration.

2.2 Antimicrobial assays

Antimicrobial assays were performed using standard protocols. Briefly, the bacterial suspension with a 10^6 CFU/ml concentration for each type of the 3 bacteria was prepared. Six replicate standard glass slides were immersed vertically in a glass tank containing 500 ml of a bacterial suspension and were incubated on a shaker at 20 rpm for 24 hours at 37˚C. After that, each glass slide was taken out from the tank using sterile forceps and was dipped twice vertically in sterile distilled water with a custom-made automated dipper apparatus under a constant speed of 0.03 m/s in order to remove loosely attached bacteria. In order to evaluate the antimicrobial effects of ozonated water, samples were vertically immersed in a tank containing 500ml freshly collected ozonated water at 25 ˚C for 1s, 5s, 10s, 30s and 1min, respectively. In this study, the LIVE/DEAD BacLightbacterial viability kit was used for the enumeration of bacteria on the glass slides. The kit consists of two nucleic acid stains: SYTO 9, which penetrates most membranes freely, and propidium iodide, which is highly charged and normally does not permeate cells but does penetrate damaged membrane. Simultaneous application of both dyes therefore results in green fluorescence of viable cell with an intact

membrane, whereas dead cells, because of a compromised membrane, show intense red fluorescence. Bacteria on samples were then stained using the LIVE/DEAD BacLightbacterial viability kit for 15 minutes and observed under the fluorescence microscope (OLYMPUS BX 41, Japan) and counted using Image Pro Plus software (Media Cybernetics, USA).

3. Result and discussion

Fig 1 shows the effect of contact time on inactivation *E. coli*. When contact time increases to 60 second (1 min), 99.9% *E. coli* were killed or only 0.1% bacteria were alive (see Fig.2).

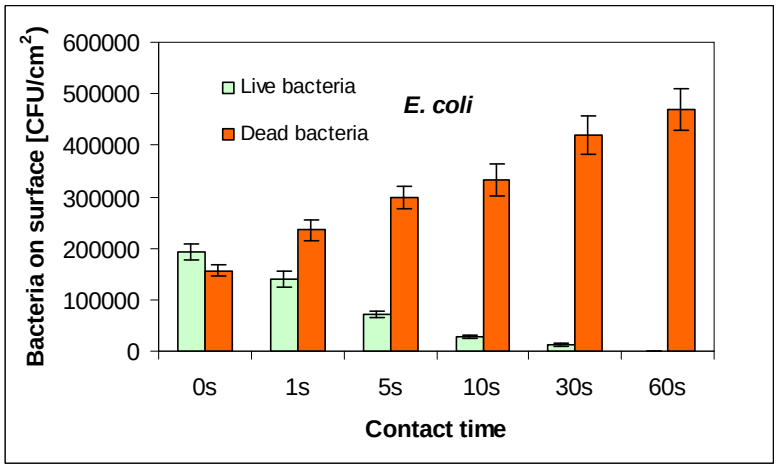


Fig 1 Effect of contact time on inactivation *E. coli*

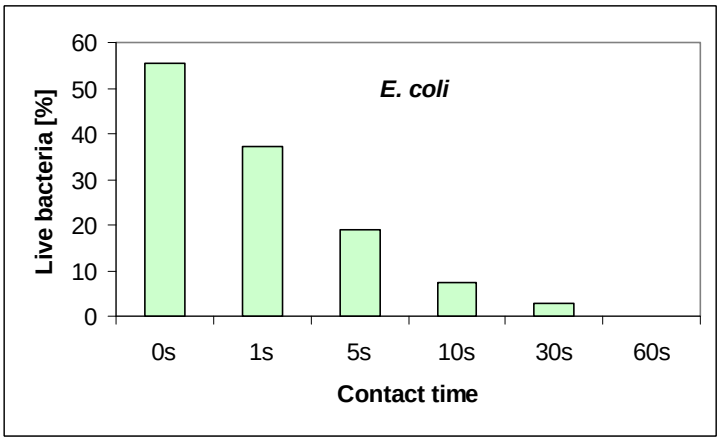


Fig 2 Effect of contact time on live *E. coli* percentage

Fig 3 shows the effect of contact time on inactivation *Staphylococcus aureus*. When contact time increases to 60 second (1 min), 99.9% *E. coli* were killed or only 0.1% bacteria were alive (see Fig.4).

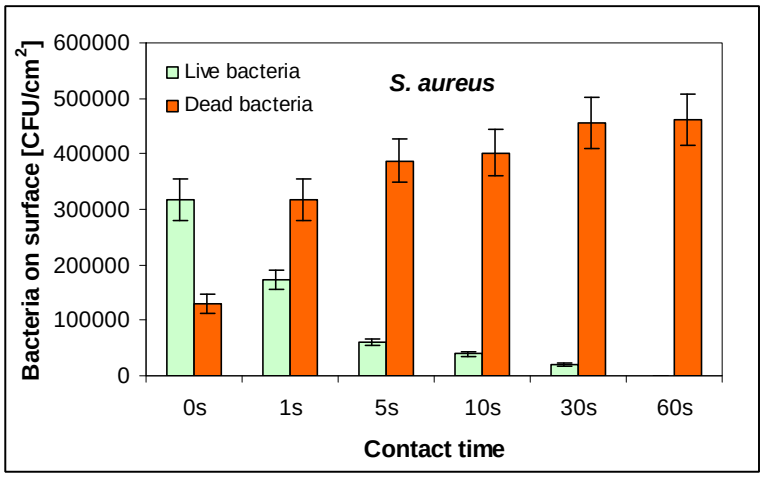


Fig 3 Effect of contact time on inactivation *S. aureus*

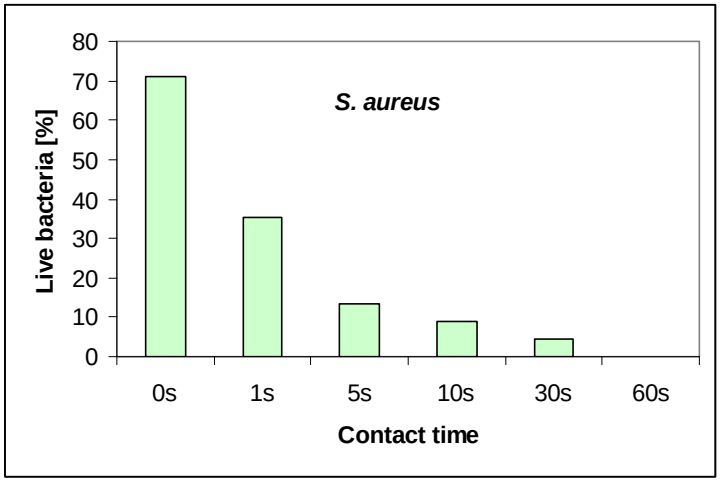


Fig 4 Effect of contact time on live *S. aureus* percentage

Fig 5 shows the effect of contact time on inactivation *Pseudomonas aeruginosa*. When contact time increases to 60 second (1 min), 99.8% *E. coli* were killed or only 0.2% bacteria were alive (see Fig.6).

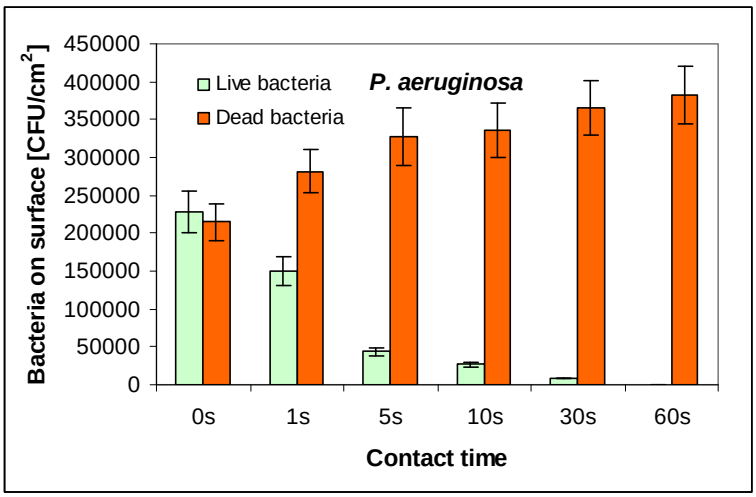


Fig 5 Effect of contact time on inactivation *P. aeruginosa*

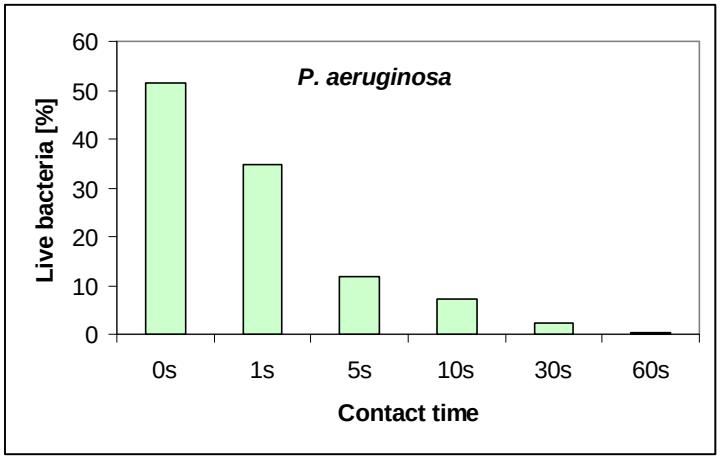
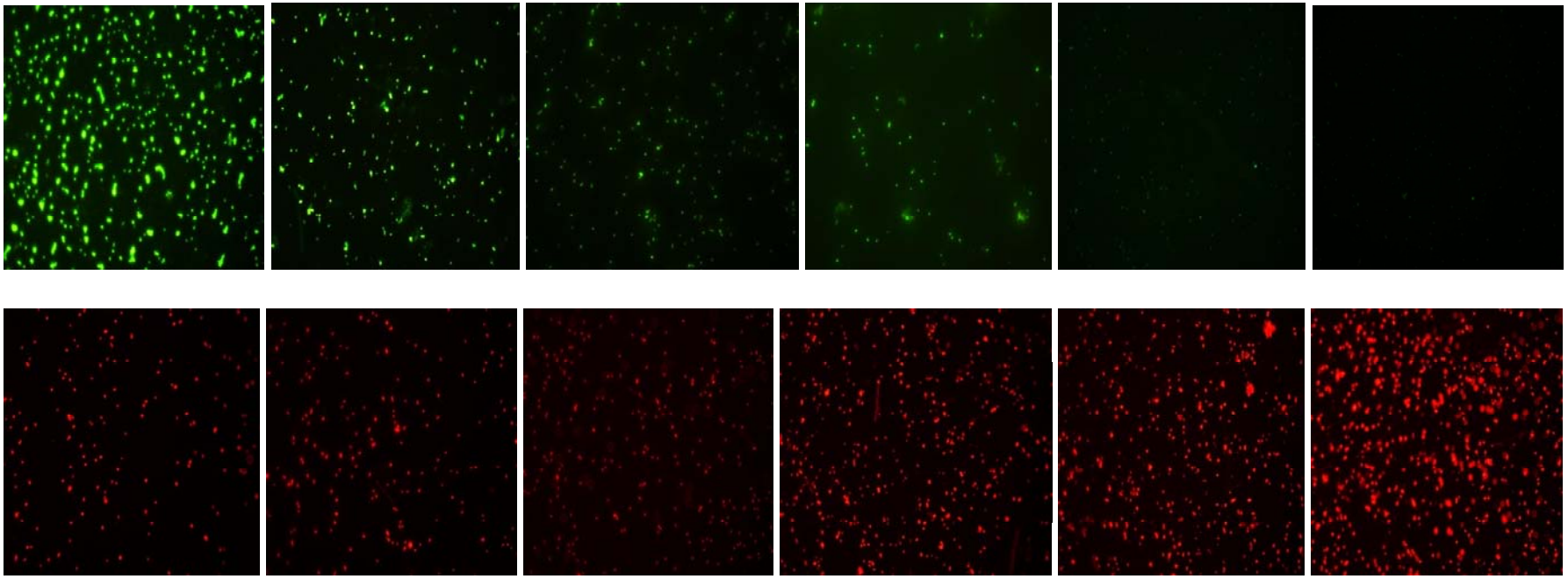


Fig 6 Effect of contact time on live *P. aeruginosa* percentage

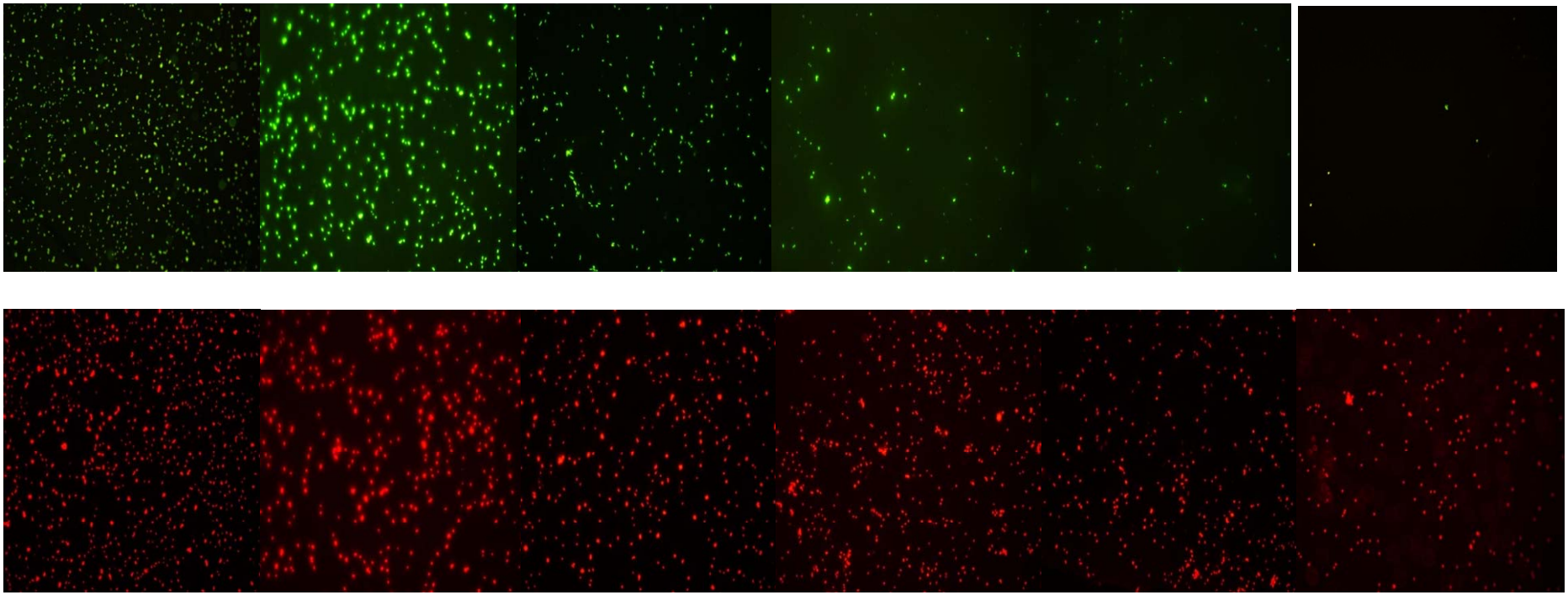
Fig. 7 shows that the typical photos of the attached live and dead *E. coli* on the standard glass slides for immersion time 24h.



(a) (b) (c) (d) (e) (f)

Figure 7 The live and dead *E. coli* on standard glass slides after immersion in ozonated water for (a) 0s; (b) 1s; (c) 5s; (d) 10s; (e) 30s; (f) 1min

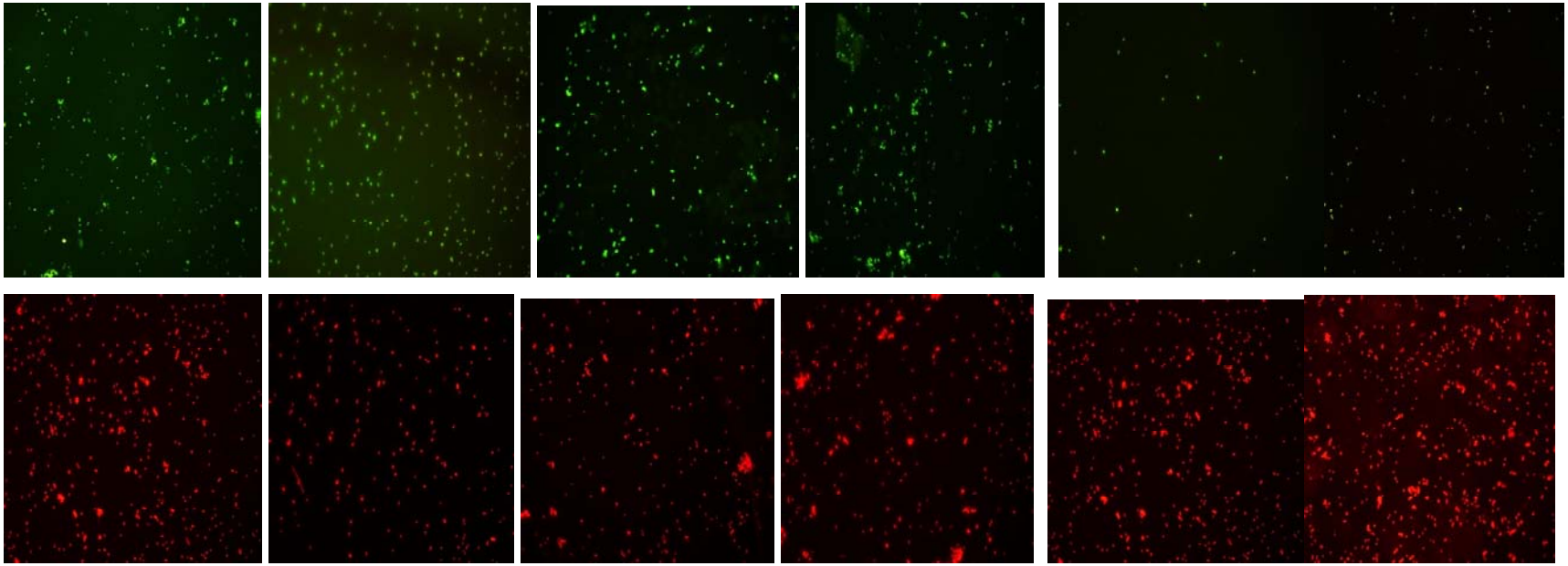
Fig. 8 shows that the typical photos of the attached live and dead *S. aureus* on the standard glass slides for immersion time 24h.



(a) (b) (c) (d) (e) (f)

Figure 8 The live and dead *S. aureus* on standard glass slides after immersion in ozonated water for (a) 0s; (b) 1s; (c) 5s; (d) 10s; (e) 30s; (f) 1min

Fig. 9 shows that the typical photos of the attached live and dead *P. aeruginosa* on the standard glass slides for immersion time 24h.



(a) (b) (c) (d) (e) (f)

Figure 9 The live and dead *P. aeruginosa* on standard glass slides after immersion in ozonated water for (a) 0s; (b) 1s; (c) 5s; (d) 10s; (e) 30s; (f) 1min

4. Conclusion

Aqualogic NT Ltd' ozonated water can inactivate bacteria on solid surfaces by over 99.8% within 1 min.