Investigating the Roles of Chlorine and Peroxynitrite Chemistry in APPJ-enabled Bacteria Inactivation

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Abstract: Atmospheric pressure plasma jets (APPJ) offer promising approaches for the inactivation of bacteria enabled by reactive oxygen and nitrogen species (RONS). This study investigates the inactivation of *Pseudomonas aeruginosa* by an RF plasma with Ar + 1% O_2. Plasma treatment produced hypochlorite compounds in saline solutions under different pH conditions. Additionally, scavenger tests confirmed the contribution of reactive nitrogen species to the inactivation.

1. Introduction

Biofilms, comprising microorganisms encased in extracellular polymeric substances (EPS), create a protective microenvironment that enhances antimicrobial resistance [1]. This property poses risks in healthcare, where biofilms are implicated in 90% of infections and 65% of hospital-acquired infections, and in water systems. EPS impedes disinfectant penetration, making biofilm inactivation challenging. APPJ treatments and plasma activated saline (PAS) provide innovative solutions by generating RONS, including long-lived species like hydrogen peroxide and hypochlorite, for safe and effective microbial inactivation [2]. However, the environmental impact of high chlorine dosages underscores the need to optimize treatment for minimal effective concentrations.

2. Methods

This study targeted *Pseudomonas aeruginosa* strain PA14 inactivation using PAS. Long-lived species were isolated by resting PAS to allow for the recombination of short-lived species before application to the bacteria. Hypochlorite concentrations were measured via an assay [3], and selected scavenger tests identified key additional inactivation contributors.

3. Results and Discussion

The results were striking, with significant inactivation observed, particularly after 60 seconds of treatment for both biofilms and planktonic phase bacteria (Fig. 1), leading to a hypochlorite compound concentration of 108 μM, a concentration much below the minimum inhibitory concentration. It is noteworthy that our investigation revealed a consistent inactivation curve across a range of pH environments. This result is attributed to the presence of a secondary disinfectant, likely enabled by peroxynitrite chemistry. The combined the effect of both H_2O_2 (a scavenger for OCl^-) and uric acid (a scavenger for $ONOO^-$) at different concentrations, support the conclusion of the peroxynitrite chemistry in addition to chlorine chemistry towards the inactivation process.

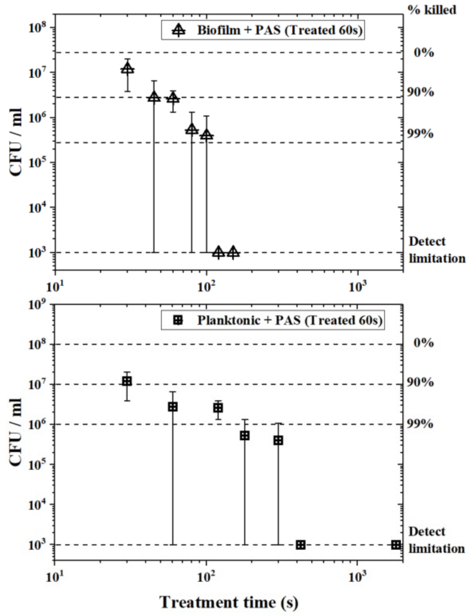

Fig. 1. Biofilm and planktonic phase bacteria inactivation by 60 seconds plasma-treated saline. Bacteria initial concentrations were set at 2.7×10^7 CFU/ml and 1.1×10^8 CFU/ml, respectively. Each datapoint contains 3 to 6 repeats of inactivation experiments.

4. Conclusion

In summary, plasma-enabled disinfection offers a multifaceted strategy to combat biofilms effectively, safely, and sustainably. This approach has the potential to address biofilm-related challenges in various fields, ranging from healthcare to environmental management, with promising outcomes and reduced environmental impact.

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References

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