Eradication of single- and mixed-species biofilms of *P. aeruginosa* and *S. aureus* by atmospheric air pulsed streamer corona discharge

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Cold atmospheric plasma has recently gained much attention due to its antimicrobial effects. Among others, plasma has proven its potential to combat microbial biofilms. Yet, knowledge of complex network interactions between individual microbial species in natural infection environments of the biofilm as well as plasma–biofilm inactivation pathways is limited. This study reports on plasma generated gaseous reactive species and induced effects on single- and mixed-species biofilms of *P. aeruginosa* (Gram-negative, G-) and *S. aureus* (Gram-positive, G+), as well as their potential eradication mechanisms. The plasma was generated by pulsed streamer corona discharge in air and used for direct treatment of biofilms. The biofilms were analyzed for bacteria inactivation, biomass reduction, metabolic activity, intracellular reactive oxygen species (ROS) accumulation, and regrowth of biofilms both in dry and wet biofilms conditions. UV-VIS emission and UV-VIS-IR absorption spectroscopy were used to identify excited atomic and molecular states and detection of reactive oxygen and nitrogen species (RONS), including nitric oxide NO, nitrogen dioxide NO_2, ozone O_3, and nitrous acid HNO_2.

With respect to biofilm inactivation single-species biofilms *S. aureus* were found more tolerant to plasma than *P. aeruginosa*, due to different cell wall structure and biofilm matrix affecting the penetration of long-lived RONS produced by plasma. Mixed-species biofilms displayed higher tolerance to plasma than single-species biofilms. No significant difference in the reduction of initial biofilm biomass was observed, although single-species biofilm of *S. aureus* has a competitive advantage over *P. aeruginosa* in terms of aggregation, attachment, and mechanical strength. Plasma also caused changes in intracellular metabolism of bacteria and led to a significant reduction of metabolic activity of cells. For *S. aureus* a reduction showed a good correlation with the bacterial inactivation. On contrary for *P. aeruginosa* many metabolically active cells were detected despite total bacterial inactivation as a result of a viable but non-culturable (VBNC) state because of oxidative stress from RONS. Relative overall intracellular ROS concentrations of bacteria in biofilms shown completely different response of G- and G+ bacteria to plasma treatment. While *P. aeruginosa* (G-) accumulated ROS inside cells followed by its leakage, *S. aureus* (G+) maintained the redox balance of cells since intracellular ROS level did not change with plasma treatment. In numbers, for *P. aeruginosa* 8 log reduction was reached and biofilm biomass rapidly reduced by 66 % for 90 s treatment, while for *S. aureus* 3 log reduction was reached and biofilm biomass reduced by 62 % for 120 s treatment, while preserving intracellular redox balance over the treatment. Despite the strong immediate inactivation, regrowth of biofilms on the next day was observed indicating inadequacy of the plasma treatment alone. Mechanical washing of cellular and extracellular debris might be still needed after plasma treatment. These results prove the importance of the total cell population inactivation inside biofilm for preventing bacteria dissemination and sustaining an infection-free surface. A comparison between wet and dehydrated biofilms revealed reduced plasma efficacy in wet environments. Dehydration of biofilms before plasma treatment facilitated the penetration of RONS in the bulk of biofilm, therefore, displaying higher inactivation. Our findings provide distinct insights into the anti-biofilm effects of plasma depending on type of bacteria and type of biofilm.

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References

[1] A. Lavrikova, M. Janda, H. Bujdakova, K. Hensel, *Sci. Total Environ.,* 959, 178184 (2025).

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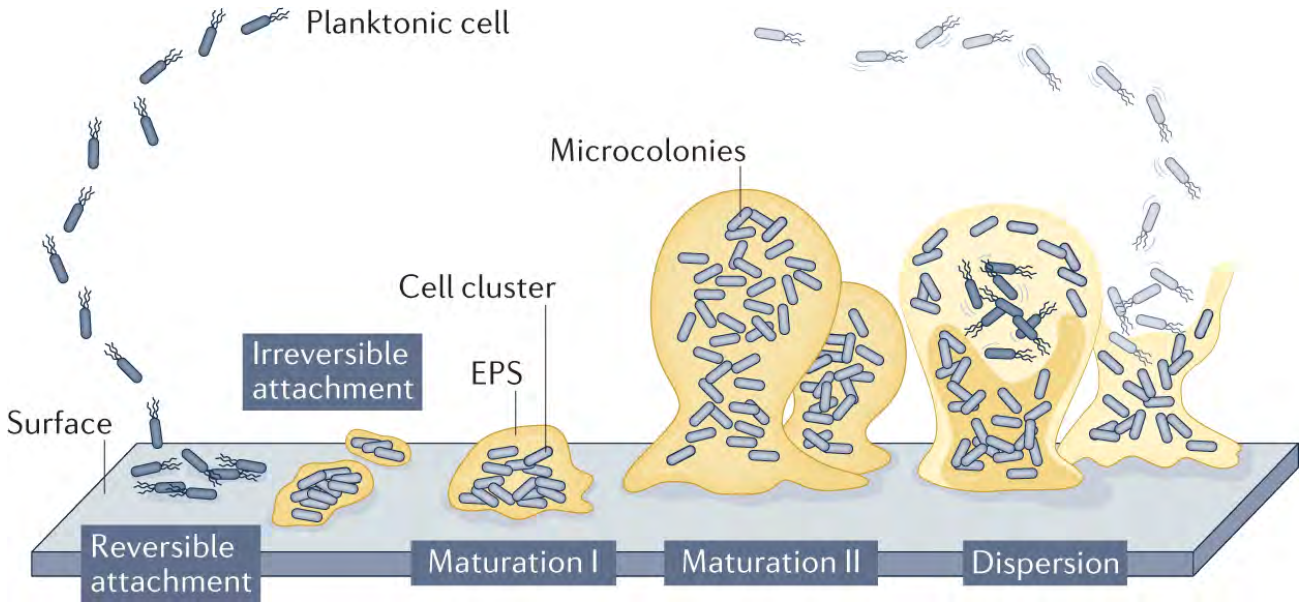
Introduction



K. Sauer, Nat. Rev. Microbiol. (2022)

Strategy	Surface modification	Microbicidal action	Biofilm destruction
Goal	Prevent biofilm formation	Inhibit biofilm formation	Eradicate mature biofilm

Biofilm control strategies

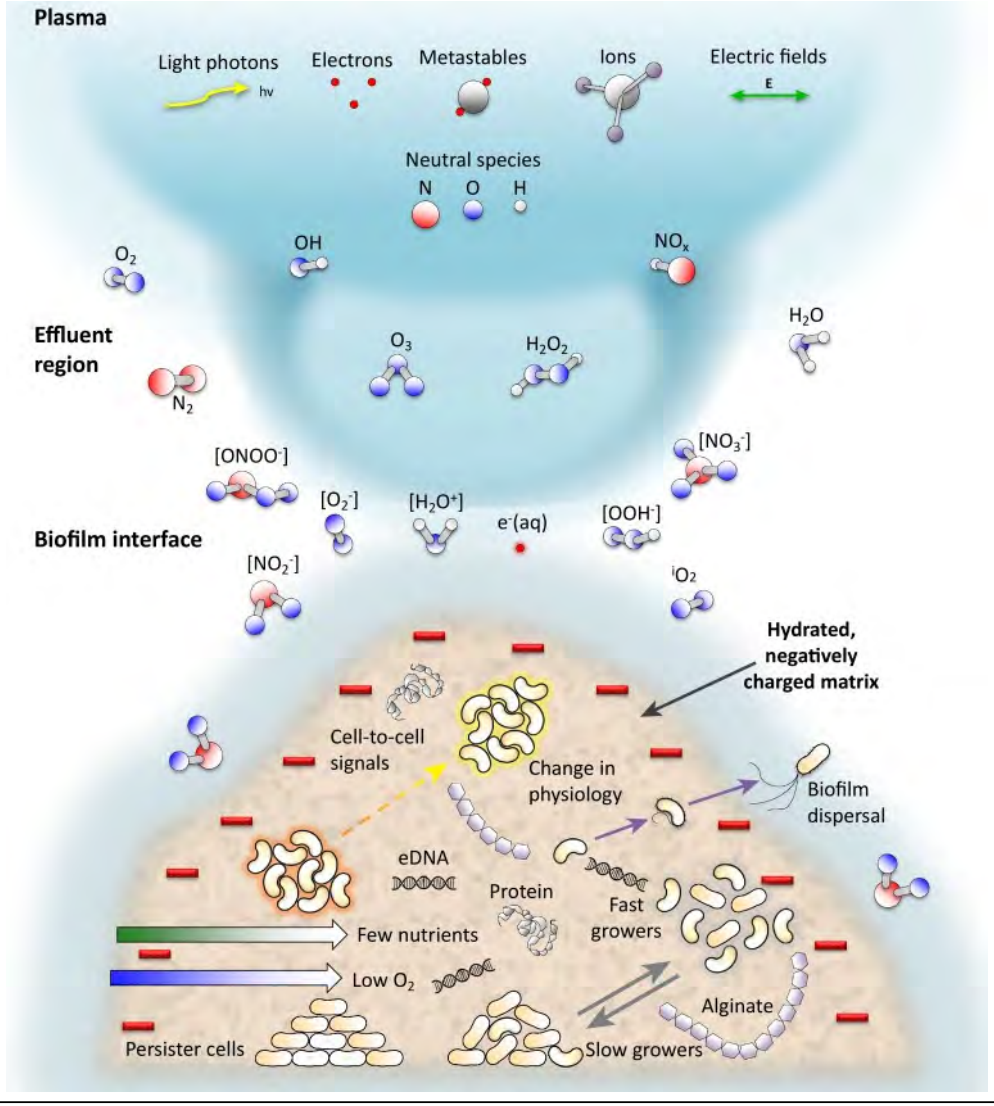
Chemical techniques

- surfactants
- antibiotics
- natural compounds

Physical techniques

- ultrasound
- irradiation
- magnetic fields
- electric fields
- other (e.g. pH or T°)
- plasma

Motivation



Antibiofilm effects of plasma

- oxidative damage
- etching
- regulation of quorum sensing

Important aspects of plasma treatment

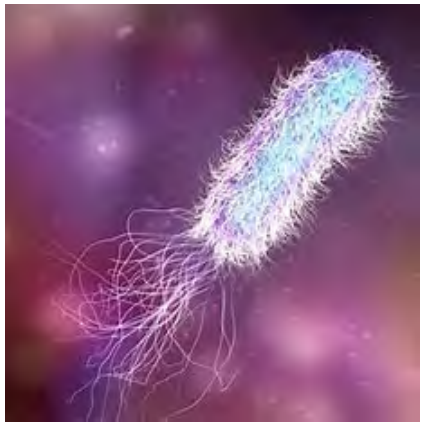
- mixed-species consortia tolerance
- biofilm regrowth
- moist vs. dry biofilms

Plasma-biofilm interface

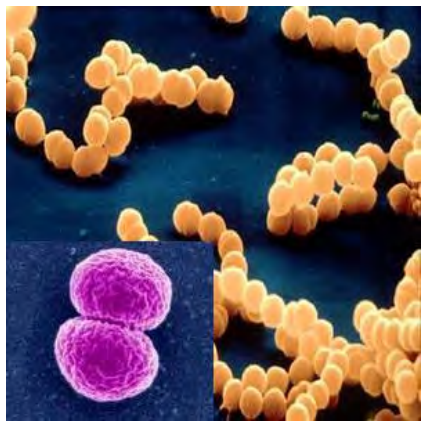
B. Gilmore, Trend Biotechnol. (2018)

Objective

- **Objective:** A closer look at the effects of atmospheric air plasma on single- and mixed- species biofilms of clinically relevant frequently co-existing pathogens *Pseudomonas aeruginosa (G-)* and *Staphylococcus aureus (G+).*
- The effect of the cold plasma generated by **pulsed streamer corona discharge** operated in atmospheric air on gaseous reactive species formation, as well as **induced effects on bacterial biofilms** (bacteria inactivation, biomass reduction, metabolic activity, intracellular ROS accumulation, and regrowth of biofilms, both in dry and wet conditions).



Pseudomonas aeruginosa

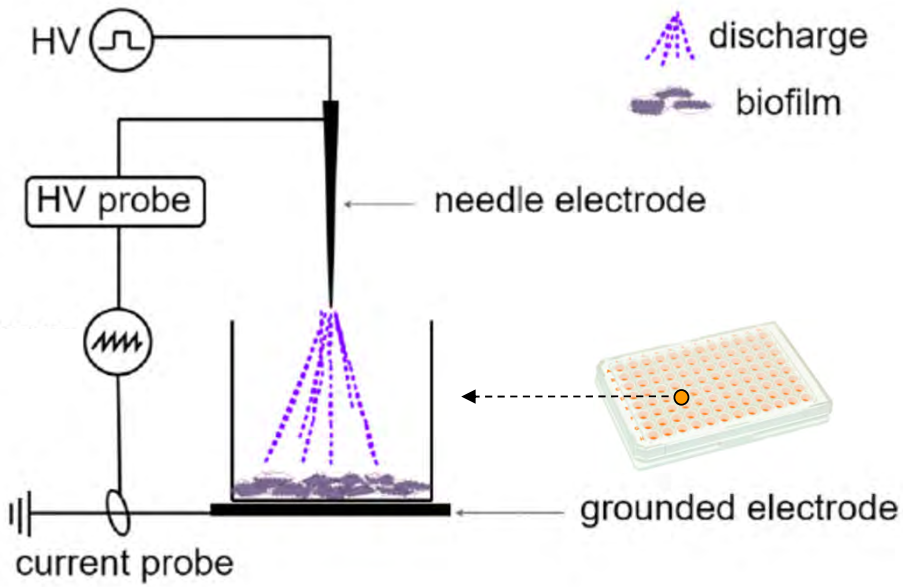


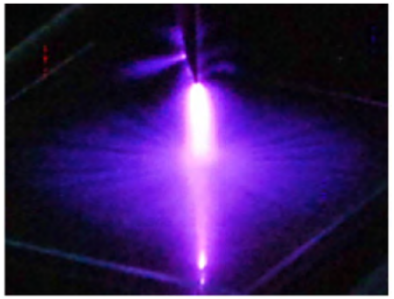
Staphylococcus aureus

A. Lavrikova, M. Janda,H. Bujdakova, K. Hensel: *Eradication of single- and mixed-species biofilms of P. aeruginosa and S. aureus by pulsed streamer corona discharge cold atmospheric plasma,* **Sci. Total Environ. 959 (2025)**

A. Lavrikova, N. C. Teja Dadi, H. Bujdáková, K. Hensel: *Inactivation pathways of E. coli and S. aureus induced by transient spark discharge in liquids*, **Plasma Process. Polym. 21 (2024)**

Material and Methods

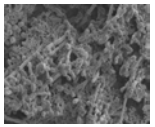




Biofilm types

Staphylococcus aureus

Pseudomonas aeruginosa



Mixed

Gas analysis

- UV-vis emission spectroscopy
- UV-vis-IR absorption spectroscopy

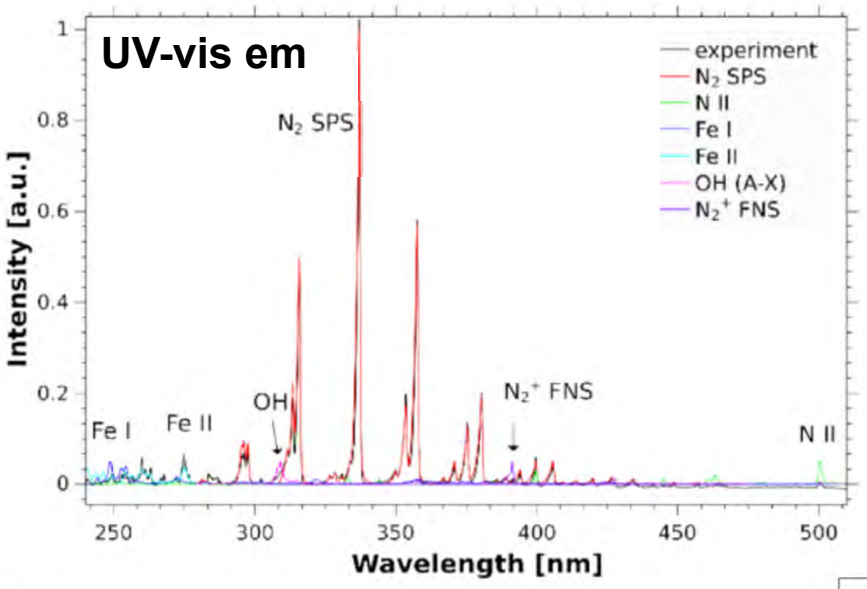
Discharge setup specification

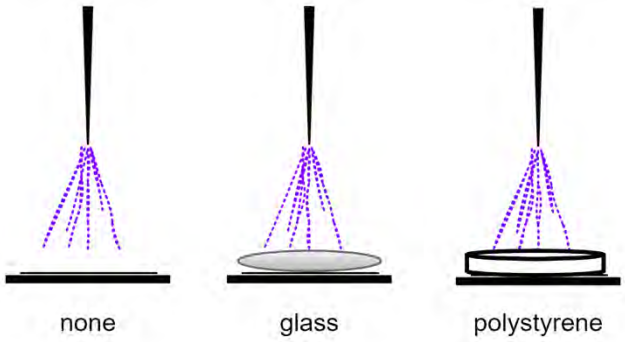
- discharge type: pulsed streamer corona
- discharge parameters: 10 kV pulse, 1 kHz
- gas conditions: ambient air, RH 45%
- needle-to-biofilm distance: 11 mm
- biofilm: on a bottom of 96-well plate, air-dried for 60 min, matured in 24h

Biofilm analysis and method of evaluation

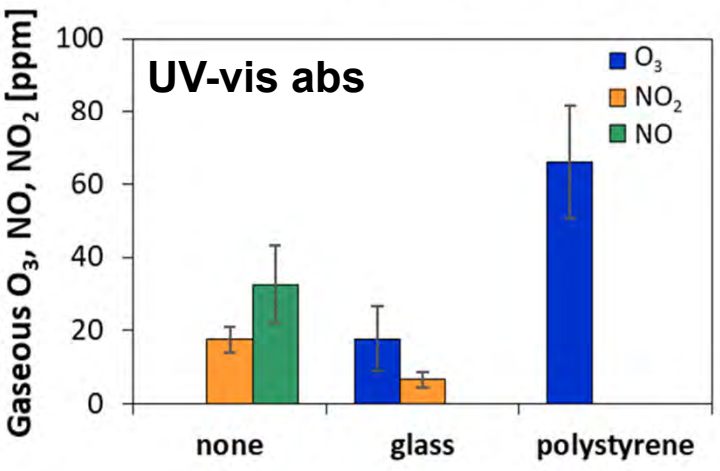
❶ biomass (crystal violet staining, λ_{abs} 568 nm)
❷ visualization (optotracer assay, λ_{ex} 550 nm, λ_{em} 660 nm)
❸ bacterial viability (CFU enumeration, LB agar)
❹ metabolic activity (MTT reduction assay, λ_{abs} 570 nm)
❺ intercellular ROS (H_2DCFDA staining, λ_{ex} 488, λ_{em} 526 nm)
❻ regrowth of biofilms (24h after plasma treatment)
❼ wet vs. dry biofilm

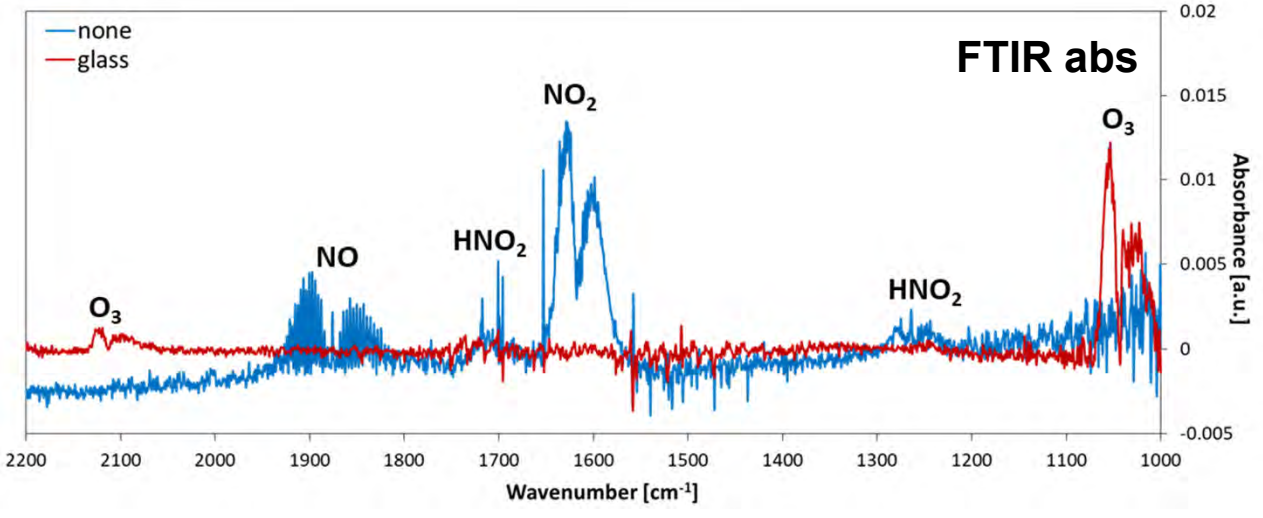
UV-vis-IR Emission/Absorption Spectra



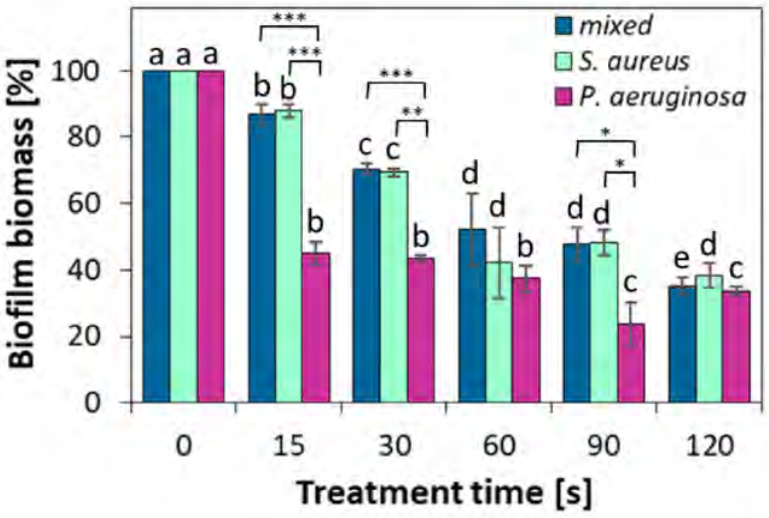


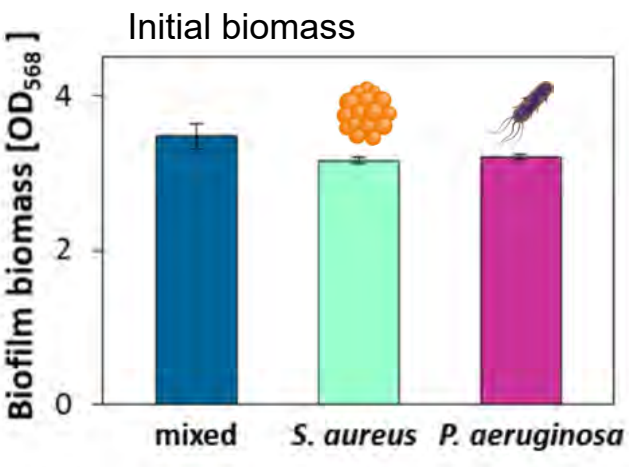
- air discharge was dominated by NO and NO_2
- with substrates on electrode (glass, PS) O_3 dominated

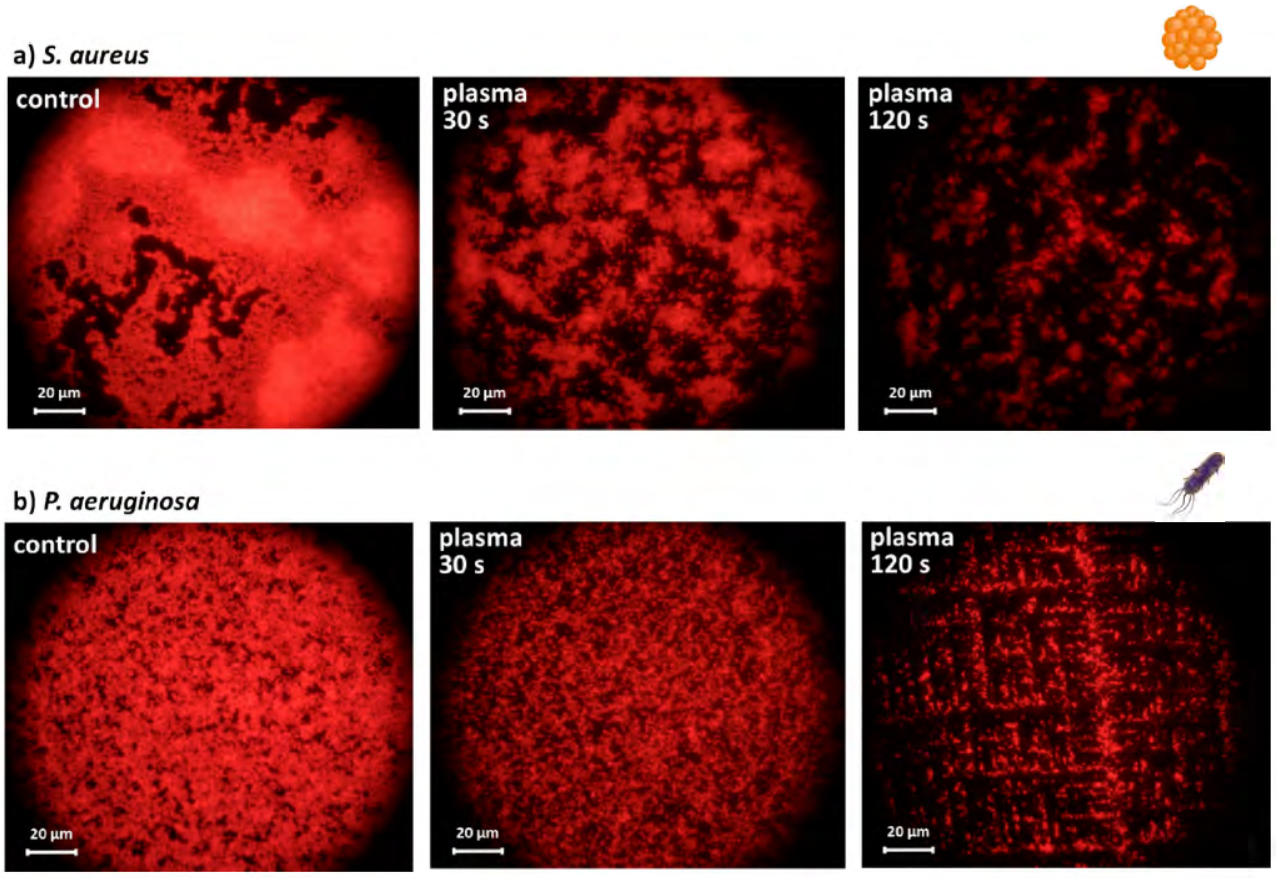




❶ Biofilm Biomass and ❷ Visualization

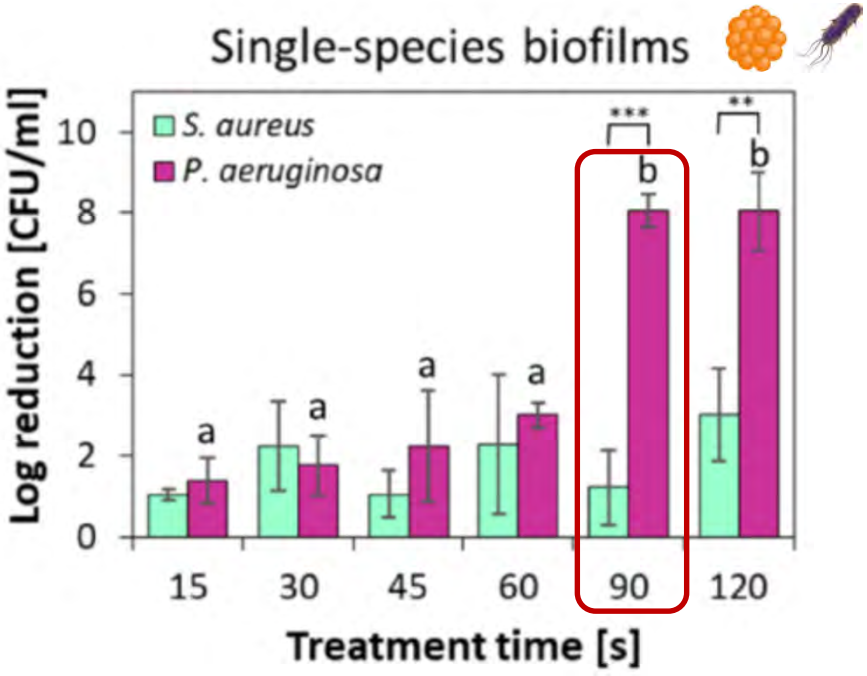


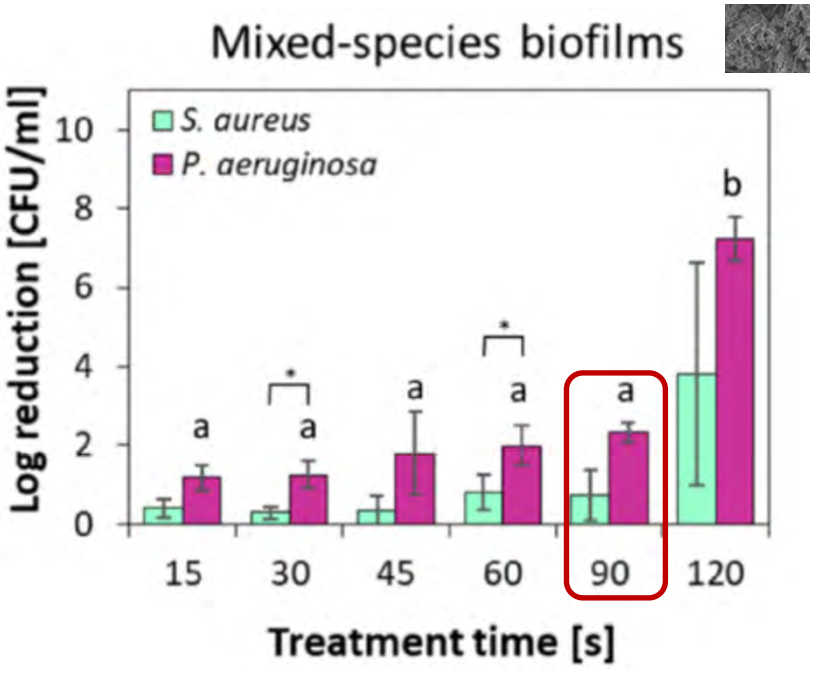




- *P. aeruginosa* – rapidly lost around 55% of its biomass
- *S. aureus* and *mixed* – endured gradual biomass reduction

❸ Bacterial Viability

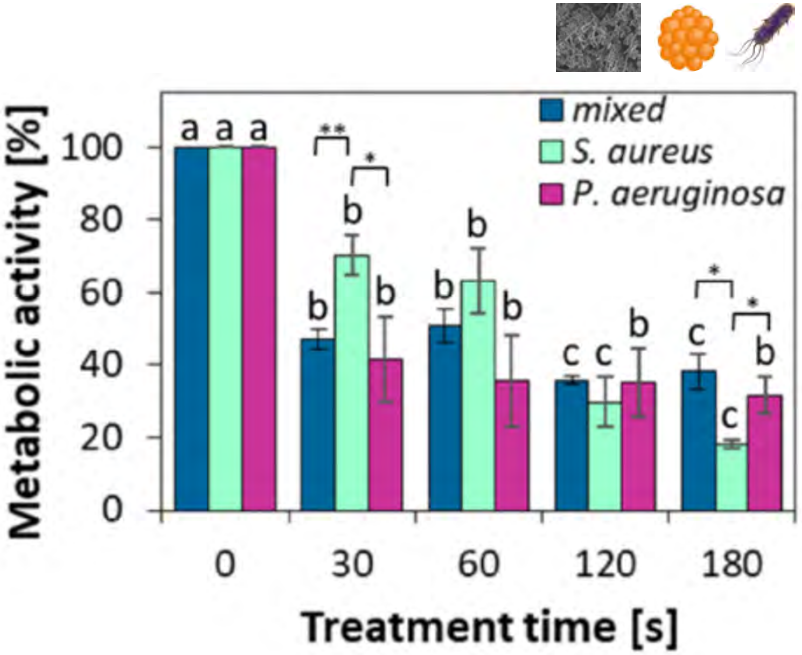


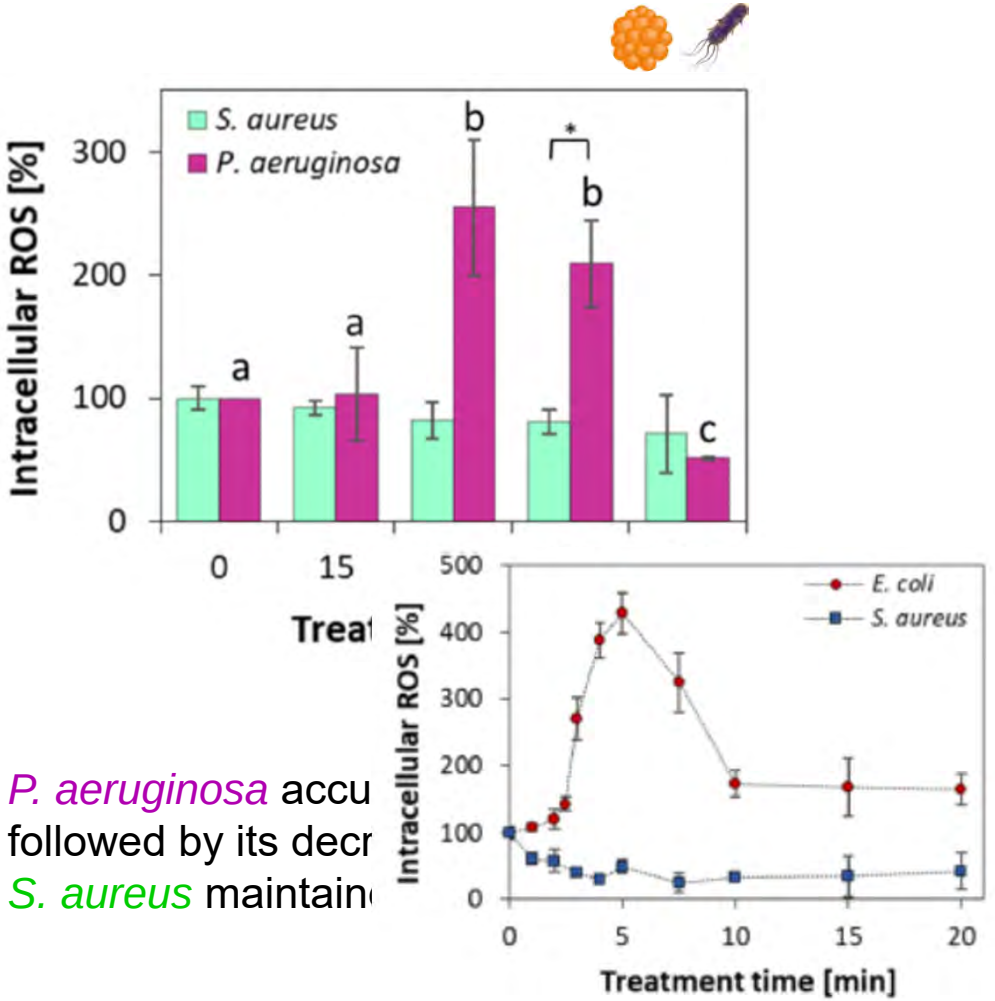


- *P. aeruginosa* – total inactivation in 90 s
- *S. aureus* – 3.2 log reduction in 120 s
- plasma eradicated the superficial layer of *P. aeruginosa* biofilm faster than of *S. aureus*

- coperative interactions in *mixed-* species biofilms led to higher tolerance to plasma of both bacteria

❹ Metabolic Activity and ❺ Intracellular ROS



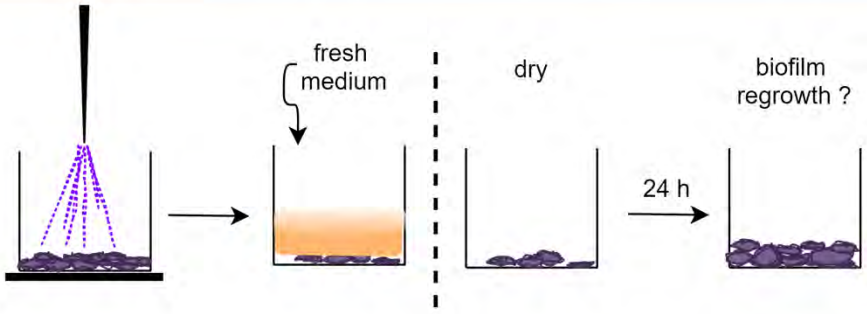


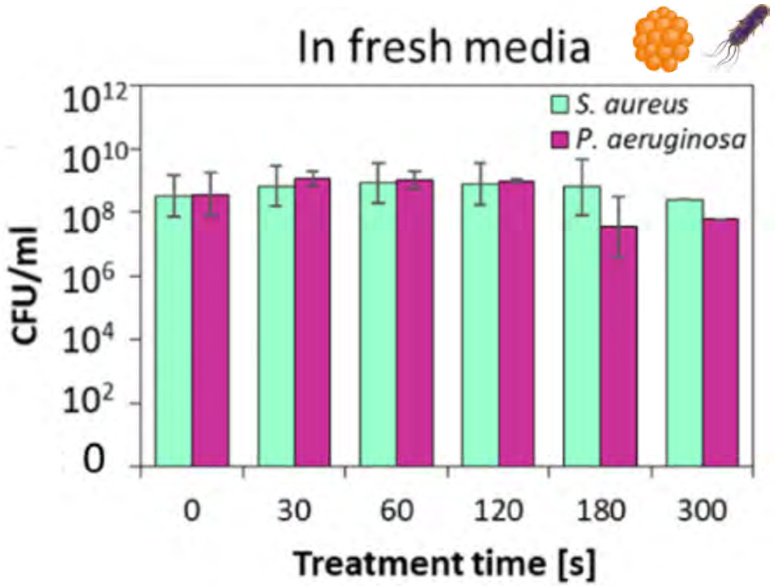
- significantly reduced metabolic activities of cells in biofilms.
- *S. aureus* – good correlation with inactivation
- *P. aeruginosa* – viable but non-culturable state

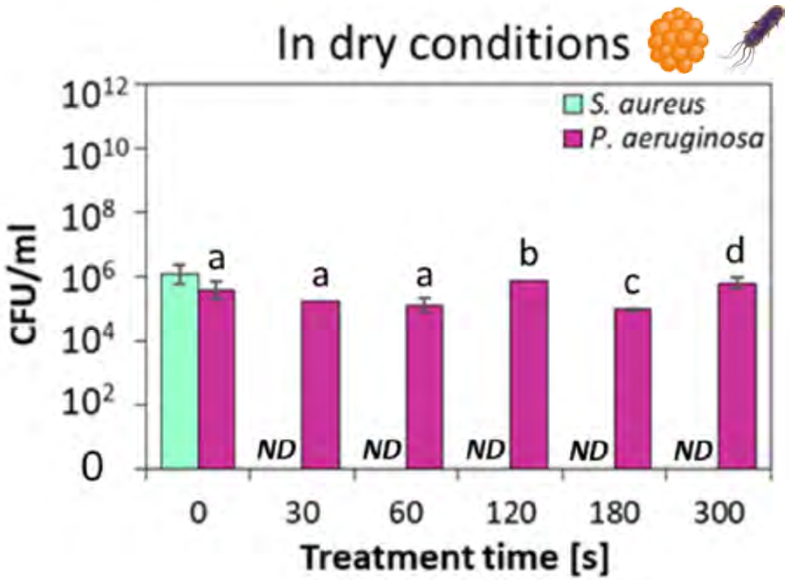
- *P. aeruginosa* accu
  followed by its decr
- *S. aureus* maintain

A. Lavrikova, Plasma Process Polym. (2024)

❻ Regrowth of Biofilms



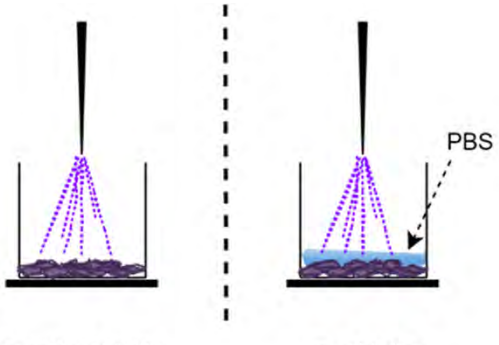




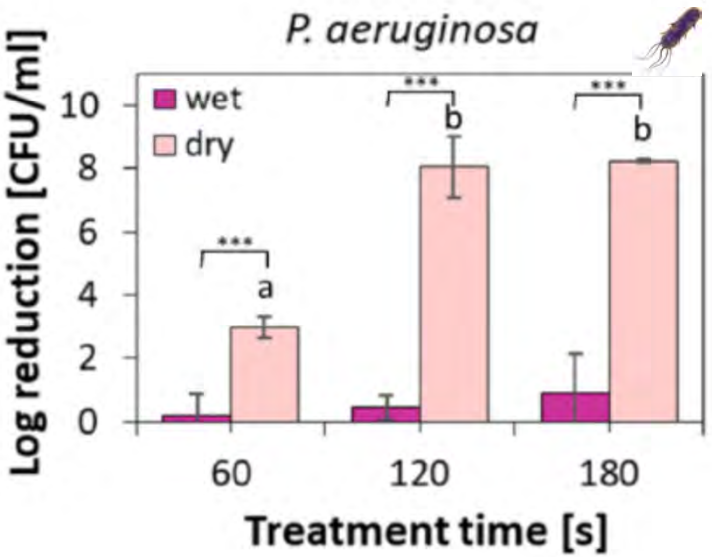
- *S. aureus* – no bacterial or biofilm regrowth.
- *P. aeruginosa* – new biofilms on the next day ⟶ inadequacy of the used plasma treatment

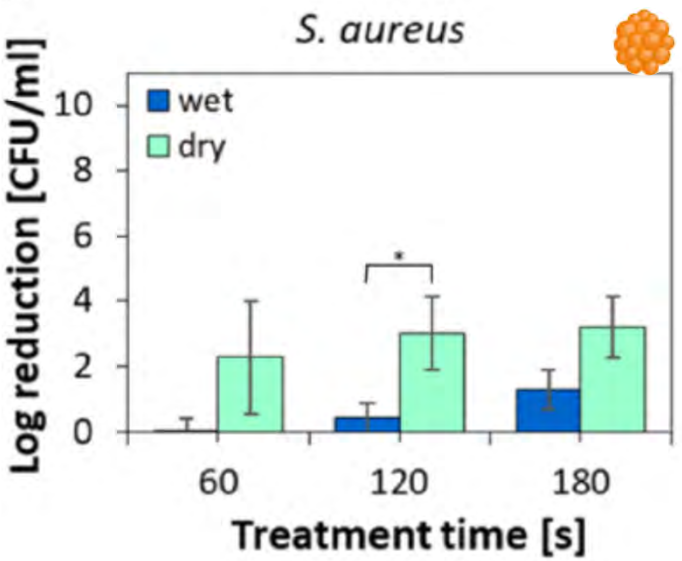
❼ Dry and Wet Biofilms

dry (dehydration for 60 min)



wet (covered with 10 µl PBS)

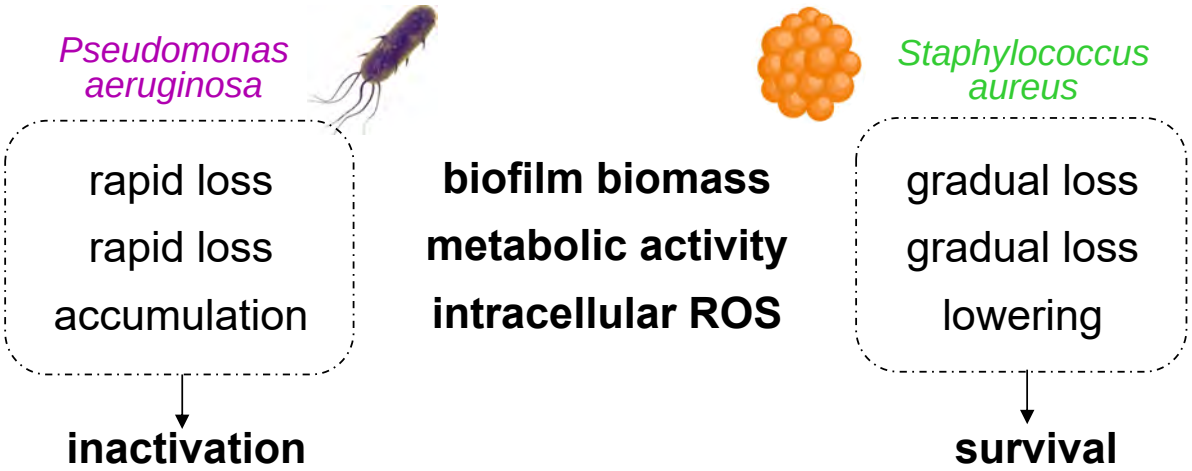




- higher tolerance of wet compared to dry biofilms for both bacteria

Conclusions

- The study reported on reactive species and induced effects on **single- and mixed-species biofilms** of ***S. aureus*** and ***P. aeruginosa***, as well as their potential eradication mechanisms.
- **Biofilms were analyzed** for bacteria inactivation, biomass reduction, metabolic activity, intracellular ROS, biofilm regrowth, in dry and wet conditions. Emission/absorption spectroscopy was used to identify RONS.



- **Mixed- species biofilms** displayed higher tolerance of both bacteria than in single- species biofilms.
- Despite the strong immediate inactivation, **regrowth of biofilms** on the next day was observed.
- Higher biofilm inactivation was found for **dry-surface biofilms** than wet ones.

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