

RUHR-UNIVERSITÄT BOCHUM

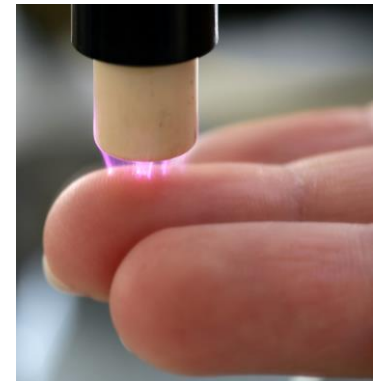
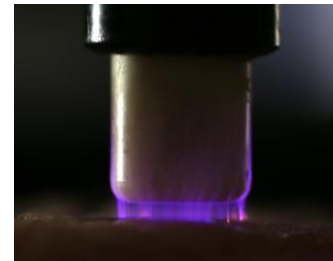
The role of humidity and UV-C emission in the inactivation of *B. subtilis* spores during atmospheric-pressure DBD treatment

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13.08.2020 - International Online Plasma Seminar

Plasma Medicine

- Plasma medicine: application of plasma devices for therapeutical usage
- Possible applications:
 - Supplement to standard wound care
 - Therapy of skin diseases (psoriasis, acne, etc.)
 - Coagulation of tissue
 - Resection of tissue (tumores, etc.)
- Focus of research: study of the impact of plasma on biological cells and tissue
 - Does plasma medicine offer an alternative to conventional medicine?



Plasma vs. Conventional Medicine?



Effects induced by treatment with non-thermal atmospheric-pressure plasmas:

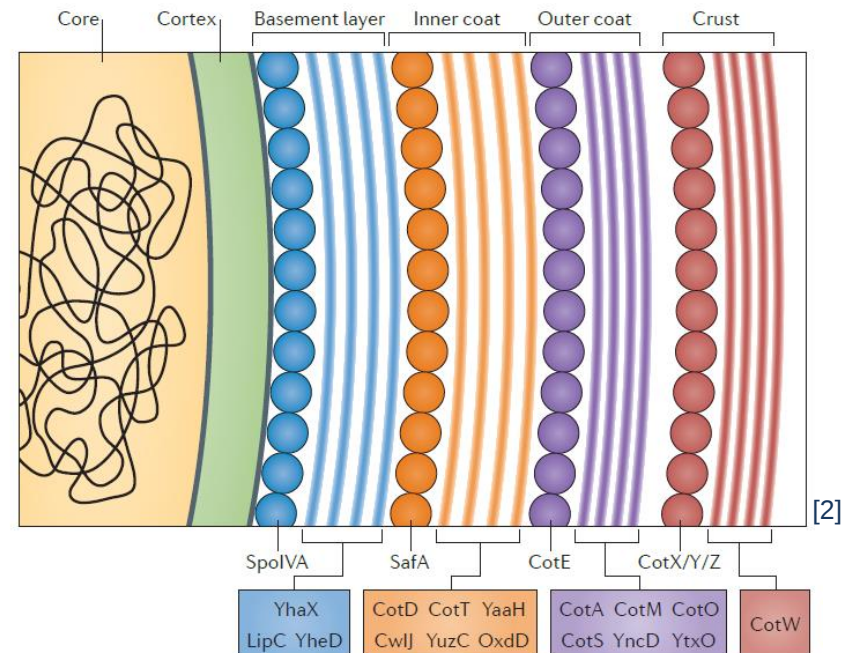
- Reduction in number of microorganisms in human wounds
- Reduction in wound size
- Enhanced proliferation

Meta-analysis of Assadian *et al.* claims:

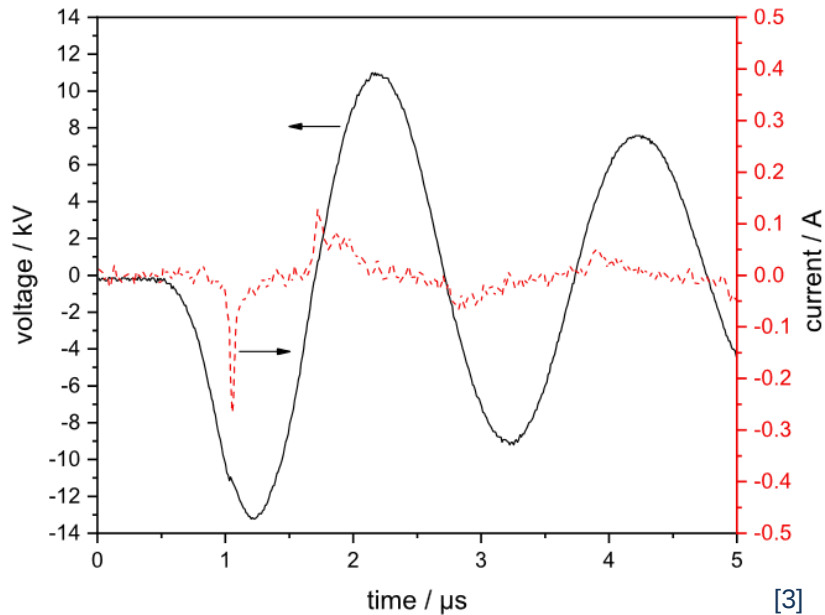
- “Use of AP in wound care is safe, but the retrieved evidence and meta-analysis show that there is no clinical benefit of AP in chronic open wounds using currently available AP device settings.”

Bacterial Spores

- Spores are formed in times of nutrient depletion
- Dormant, long-living form to preserve the genetic information of the cell
- Spore coat gives protection to reactive species, predators and UV-radiation
- *B. subtilis* as a common used bioindicator for sterility due to high resistance to outer stressors

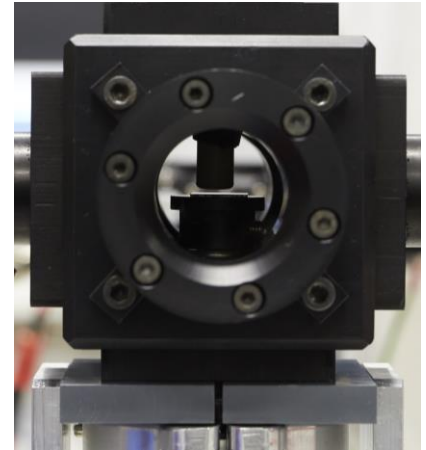


Experimental Setup



Treatment conditions:

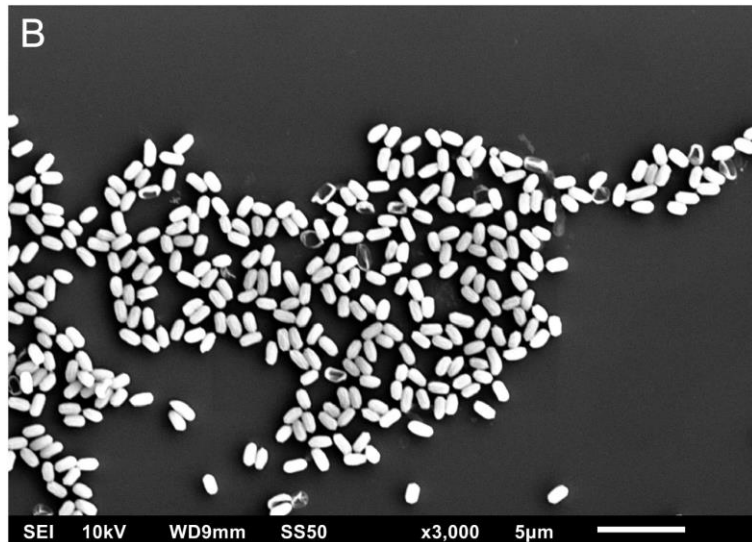
- $f = 1000 \text{ Hz}$, $V_{\text{pp}} = 24 \text{ kV}_{\text{pp}}$
- Process gas:
 - Ambient air
 - N_2/O_2 mixtures
 - Synthetic air with relative humidity
- Counter electrode: glass slide with sprayed spores on grounded aluminum plate



Plasma Treatment

Sample Preparation

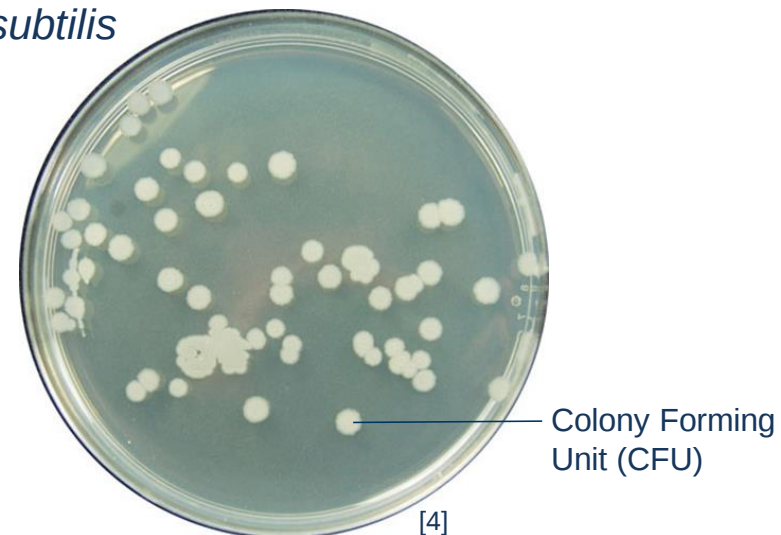
- *B. subtilis* wild type PY79



- Sprayed in a monolayer on glass slides to avoid shielding

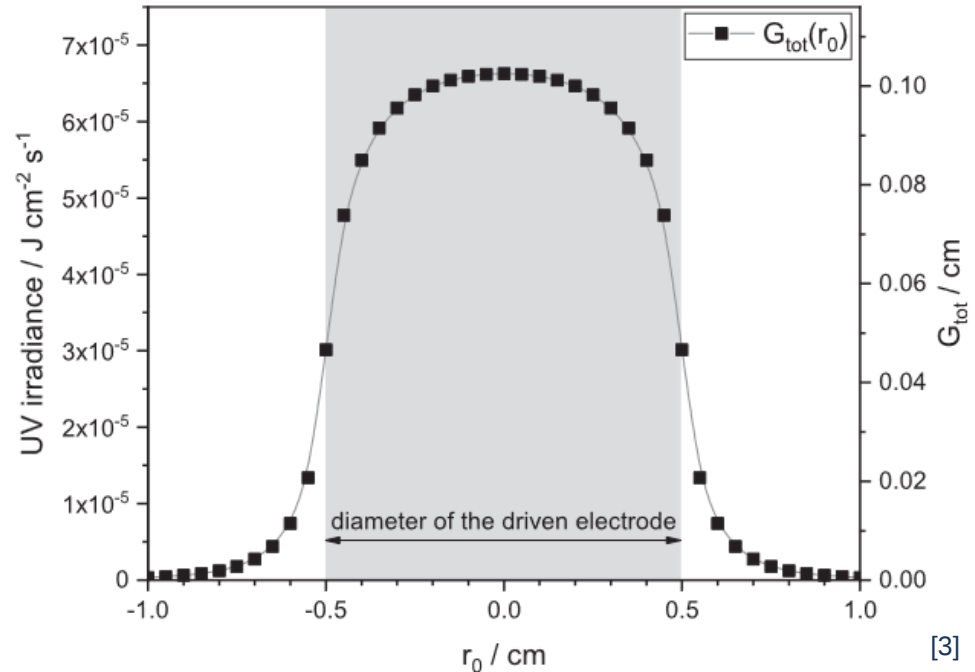
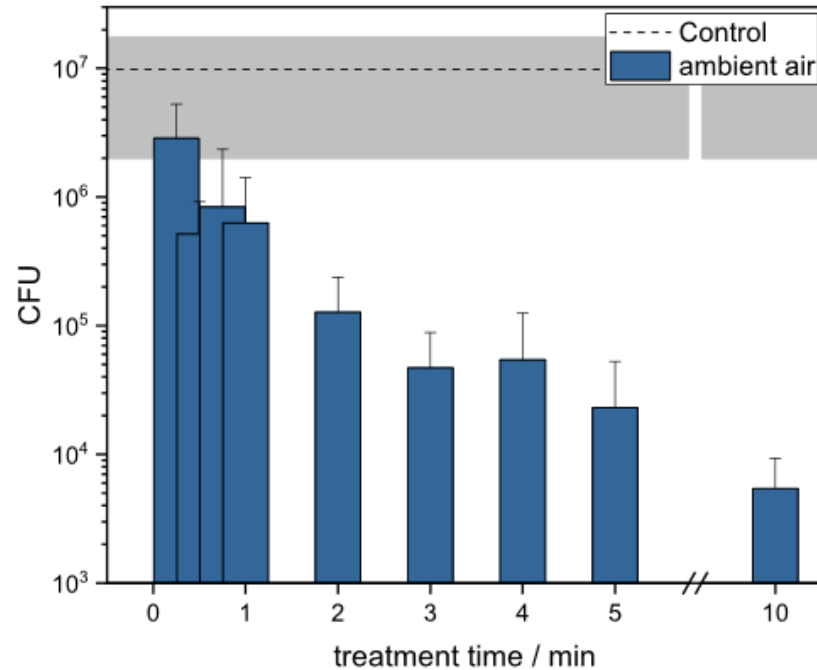
Evaluation

- Cultivated on Agar plates – outgrowth of *B. subtilis*



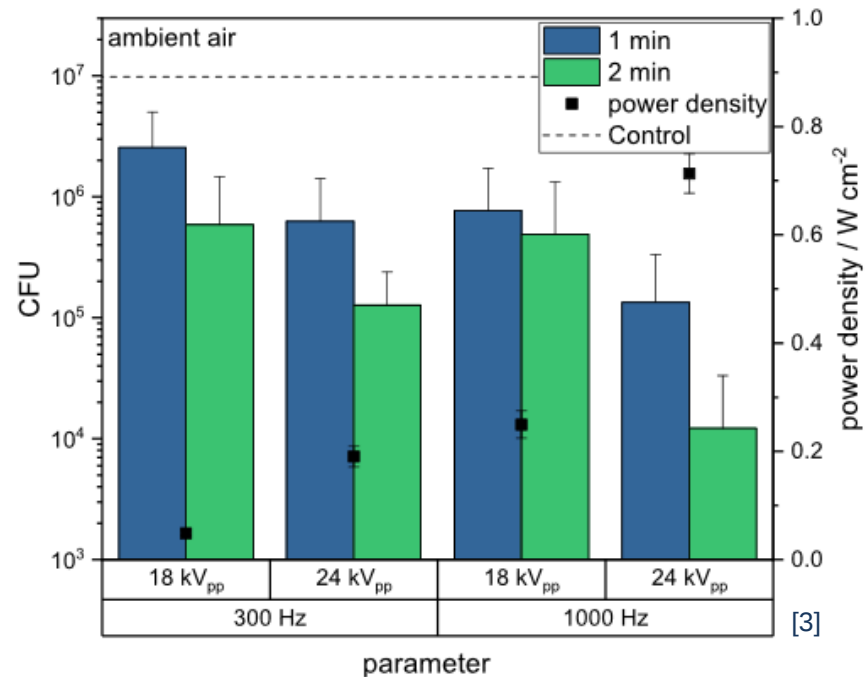
- Number of colonies represents number of surviving spores

Inactivation of spores in ambient air



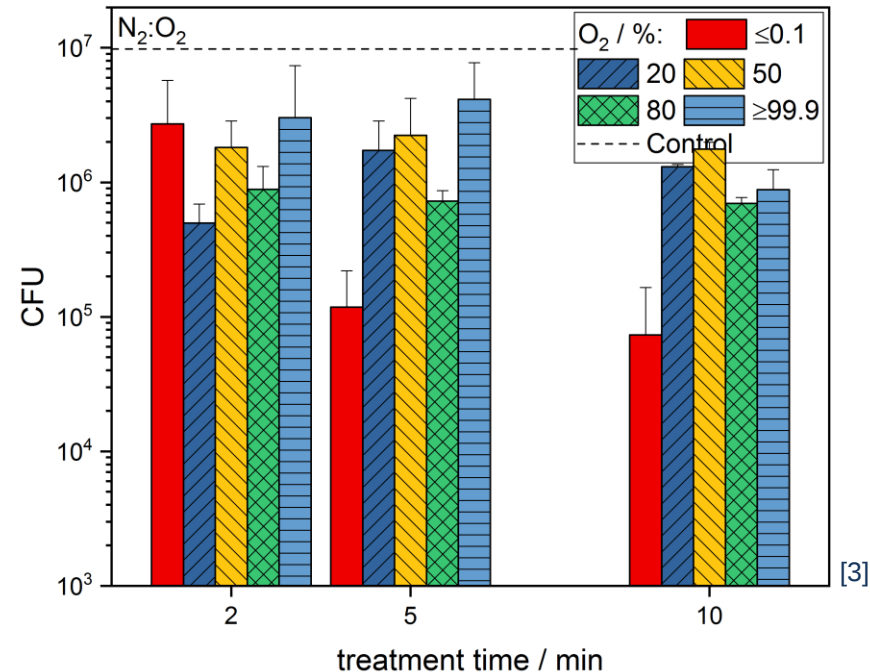
- Within 3 min: number of surviving spores reduced by two orders of magnitude
- Decrease in inactivation for longer treatment times due to spatial profile

Inactivation of spores in ambient air



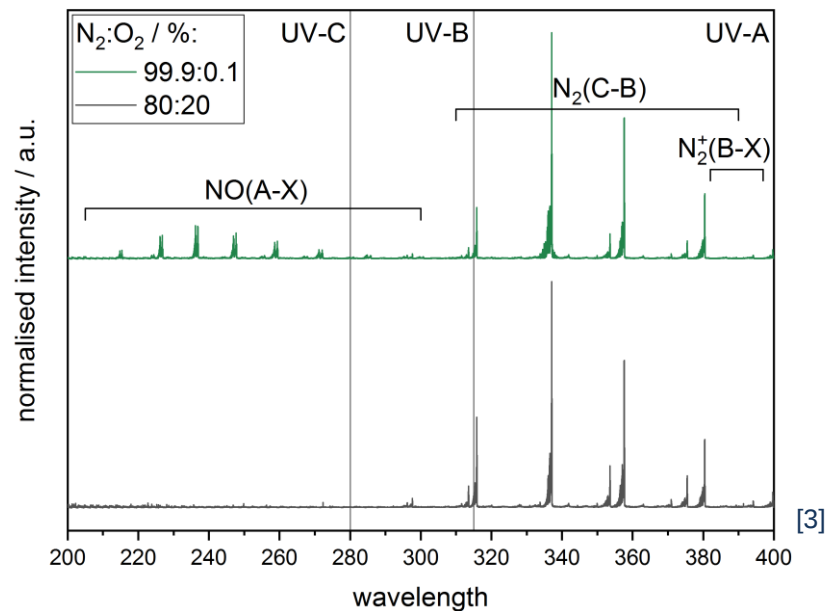
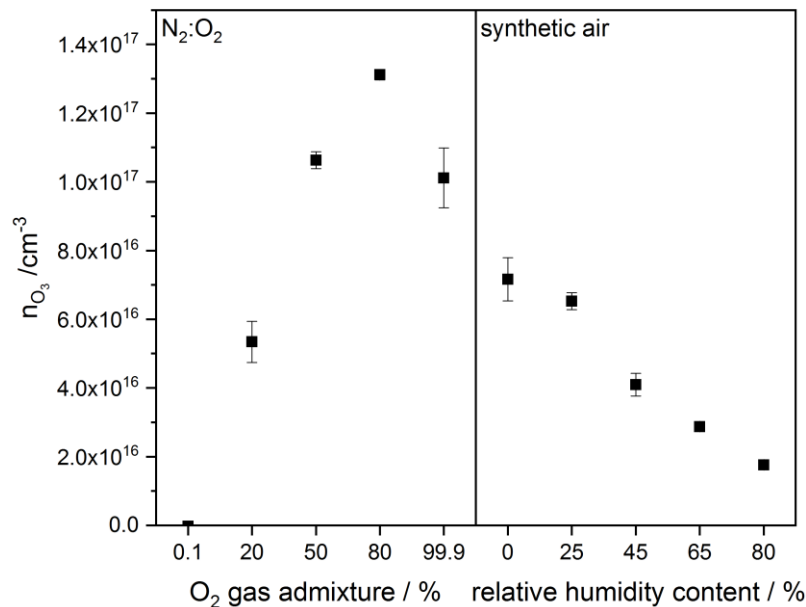
- Reduction of CFU correlates with power density

Inactivation of spores in N₂/O₂ atmosphere



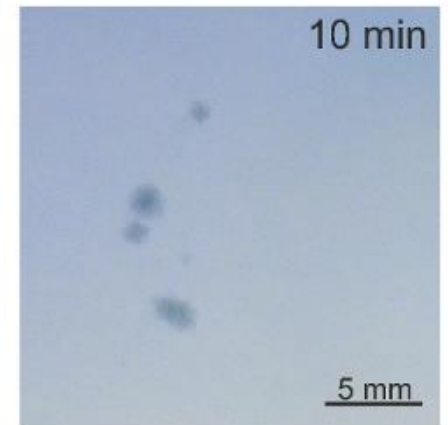
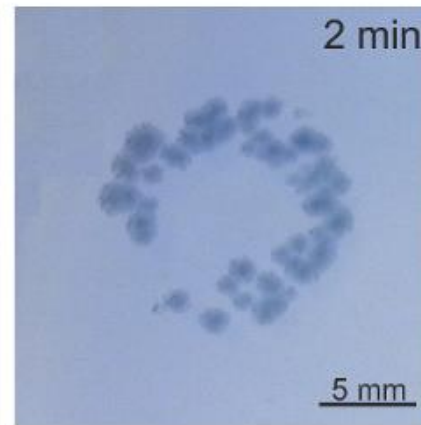
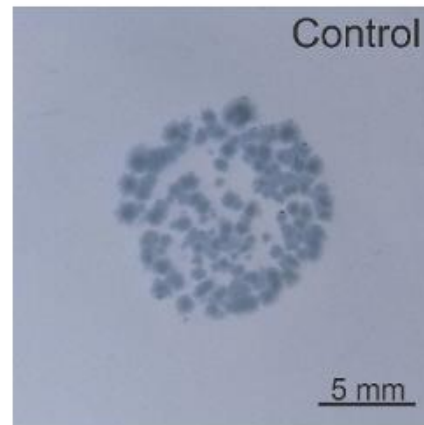
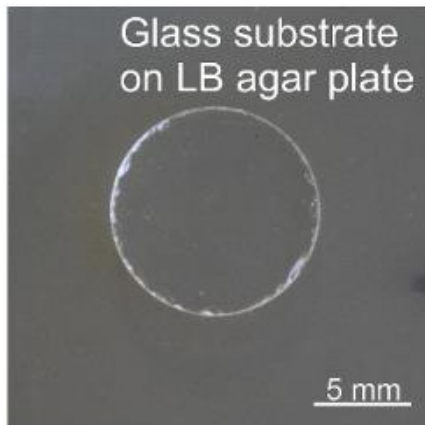
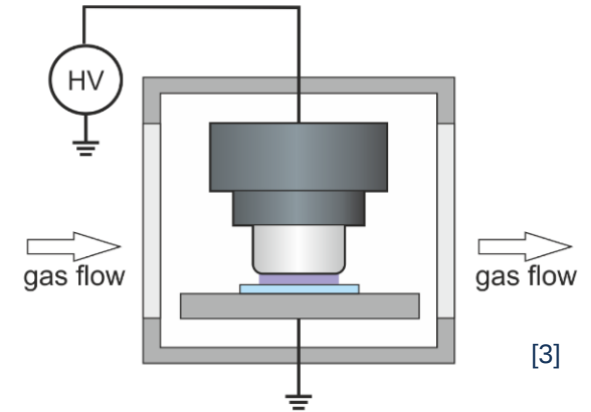
- Controlled changes in the plasma chemistry
- Strong inactivation in an atmosphere of N₂ with oxygen residuals (≤0.1%)

O₃ density and UV emission in controlled N₂/O₂ atmosphere



- Ozone, O₃: powerful oxidant
- UV emission: potentially damaging for cells (DNA-damages) due to high energies

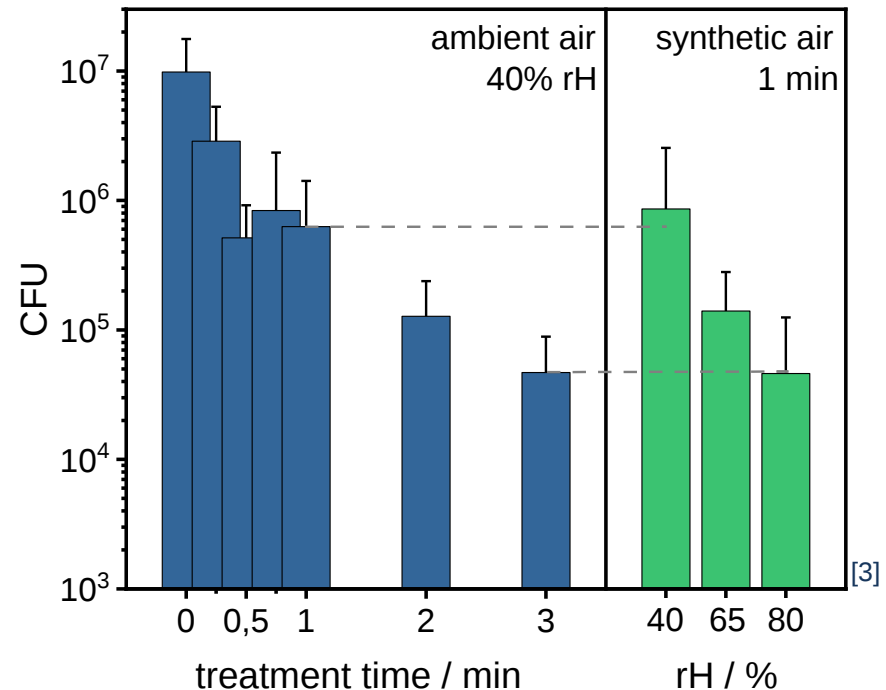
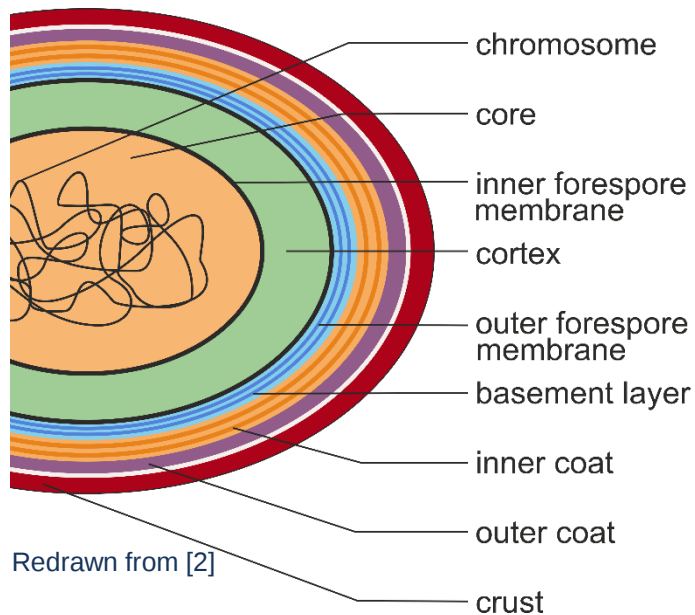
Is it all about the flux profile?



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- Increase of the area of inactivated spores in the centre of the discharge
- Influence of the flux profile clearly visible

Inactivation of spores in controlled humidity content



- Strong impact of relative humidity
- Within 1 min: number of surviving spores reduced by two orders of magnitude

Possible inactivation mechanism

- DBD vs. spores: reduction achieved!
- No positive correlation between spore inactivation and UV-A or UV-B emission
- No positive correlation between spore inactivation and ozone
- UV-C irradiance due to NO emission might play a role
- An increase in humidity leads to less surviving spores
- Reactive water-related species, such as OH or H₂O₂ might lead to inactivation of spores
- Gas-phase acids, such as HNO₂, HNO₃ or HNO₄ might have an influence

**Thank you for your
attention!**

Bibliography

- [1] F. Brehmer *et al.*, Journal of the European Academy of Dermatology and Venereology 29.1, 148-155. 2015
- [2] P. T. McKenney *et al.*, Nature Reviews Microbiology, 11.1, 33, 2013
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- [4] PP0282 – Tryptone Soya Agar. [Online]. Available: <http://www.eolabs.com/product/pp0282-tryptone-soya-agar-irradiated/>