Young Scientist Special Session

Next generation of decontamination technologies: challenging Bacillus subtilis biofilms with cold plasmas

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Biofilms are highly resistant consortia of microorganisms that are attached to a surface. These communities are embedded in a protective and self-built matrix containing extracellular polymeric substances (EPS). Due to advanced protection and resistance mechanisms, biofilm inactivation remains a challenging task, causing serious problems in almost all man-made environments. Hence, there is an urgent need for reliable sterilization approaches. Plasma, the 4th state of matter could be a promising next generation sterilization approach of biofilms. Its antimicrobial properties are achieved by a mixture of reactive oxygen and nitrogen species (RONS), excited atoms/molecules, charged particles and UV-photons.

This project aims to improve the decontamination processes of bacterial biofilms by cold plasmas. Therefore, it is crucial to uncover underlying protection mechanisms and understand interactions between individual plasma elements and biofilm structures.

Since biofilms are highly heterogeneous communities developing over time, various maturity levels were treated with hydrogen peroxide (H_2O_2). Here, H_2O_2 represents a source for reactive oxygen species (ROS) and one of the major biocidal parts formed during gas plasma discharge. Since *Bacilli* are well-investigated and used as quality control strains for conventional sterilization methods, B. subtilis was chosen as bioindicator. Lethal effects were assessed by calculating colony forming units (CFU) of spores as well as vegetative cells. Since this research addresses factors within biofilms that could contribute to ROS resistance, the impact of exopolysaccharides and sporulation was tested by using strains lacking corresponding genes (*epsA-O* and *sigG*) and were compared to B. *subtilis* NCIB3610.

In general, wildtype biofilms of all maturity levels were the most resilient to H_2O_2 treatment. Planktonic cells (0h) of the sporulation-deficient strain were not able to survive ROS exposure but since consortia are formed (24h-72h), no reduction in CFU can be detected. Biofilms without EPS survived ROS stress worse than the biofilms free of spores, regardless of the maturity degree. These results indicate that intact EPS within the matrix is more protective than sporulation. To verify whether both, sporulation and EPS are essential to survive H_2O_2 exposure, a strain lacking ability to sporulate and EPS production will be generated and tested.