Summary for the final report
Identification of suitable surrogates for Aspergillus species for the microbiological validation of UV-based disinfection processes (Surrogates)

In the food industry, UV radiation is often used to decontaminate the surfaces (e.g. packaging materials, machine parts). Microbiological validations are carried out to determine the inactivation efficiency of decontamination methods for food filling lines. They are an important tool during the development phase or in the commissioning. Special test microorganisms or microorganism spores are used for the production of artificially contaminated sample carriers. These should have a high and defined resistance against the used decontamination agent, be easily detectable and have no pathogenic properties. For the investigation of UV-based decontamination methods, conidiospores of *Aspergillus brasiliensis* DSM 1988 are recommended by VDMA (Mechanical Engineering Industry Association) as bioindicator. The use of this strain is well established and widely used in Germany and Europe as well as in many other countries.

In 2016, *Aspergillus brasiliensis* DSM 1988 was reclassified from risk level 1 to risk level 2 in the "Technical Rules for Biological Agents (TRBA 460)". The Federal Office for Occupational Safety and Health (BAuA) prohibits the use of such organisms outside laboratories of protection level-2. Consequently, *Aspergillus brasiliensis* DSM 1988 cannot be used at microbiological validations of food filling lines since the reclassification. The aim of this IGF project was to find suitable surrogates for *Aspergillus brasiliensis* DSM 1988.

Within the scope of the project, a comprehensive literature review and a resistance screening was carried out with the aim of identifying a selection of potentially suitable candidates. The cultivation and sporulation conditions of selected strains were determined and the detailed resistance investigations were carried out using UV-C lamps (Low-Pressure, Medium-Pressure lamps) as well as Xenon Pulsed-Light decontamination system. A total of 16 different molds and bacteria were tested for resistance against Xenon Pulsed-Light and 18 molds against UV-C Medium-Pressure lamp. The resistances of the respective microorganisms were compared using decimal reduction doses (DRD values).

The initial investigations with Xenon Pulsed-Light showed that the *Penicillium rubens* (formerly *P. chrysogenum*) DSM 848 and DSM 844 has very similar resistances as *A. brasiliensis* DSM 1988.

The investigations with Medium-Pressure lamp identified the strains *Aspergillus carbonarius* DSM 872, *Alternaria alternata* DSM 12633, *Ulocladium consortiale* DSM 62014 as possible surrogates. All other screened strains showed significantly lower DRD values than *A. brasiliensis* DSM 1988, so they were excluded as surrogates. However, the selected surrogate strains were found to be significantly more resistant to UV radiation than *A.*

brasiliensis DSM 1988. Various methods described in the literature were investigated to modify the UV resistance. Using of DMSO (dimethyl sulfoxide) as an inhibitor of melanin synthesis proved to be effective and resulted in a reduction of the UV-resistance of *Aspergillus carbonarius* DSM 872. The DMSO was added to the sporulation medium during cultivation, and subsequently, the resistance of the spores against UV radiation was determined. This revealed that the color of the spores became lighter and the UV resistance strongly decreased with increasing DMSO concentration in the sporulation medium. Comparison of the determined inactivation kinetics of *A. carbonarius* DSM 872 with different DMSO concentrations and *A. brasiliensis* DSM 1988 showed that the use of a DMSO concentration of 0.8 mg/g in the sporulation medium resulted in very similar UV resistances.

In further investigations with Low-Pressure lamp was detected, that *A. carbonarius* DSM 872 with concentrations between 1.0 mg/g to 1.4 mg/g DMSO in the sporulation medium had a good correlation with the inactivation kinetics of *A. brasiliensis* DSM 1988.

Additionally, following practice-relevant aspects were scientifically investigated and evaluated as part of the project:

- Storage stability of the surrogates in suspension and as bioindicators before and after the decontamination processes,
- Changes of UV resistance during the storage period,
- Optimal artificial contamination methods of the surfaces,
- Influence of the carrier material.

Based on the aforementioned studies, it was demonstrated that the spores of *P. rubens* DSM 844 did not exhibit stable behavior in terms of UV resistance or bacterial count under the test conditions.

Within the scope of the studies on the storage stability of the spore suspensions of *A. carbonarius* DSM 872 and *P. rubens* DSM 848, it was proven, that the UV resistances of the spore suspensions remain almost stable for 4 months at 4 °C in Ringer's solution.

In the investigations with different carrier materials, the inactivation of the test microorganisms at identical irradiation doses showed significantly higher inactivation rates on aluminium carrier materials than on glass and polypropylene.

In the conducted investigations, the following strains stood out for their suitable resistance, simple preparation and handling as well as their stable storage behavior under the different storage conditions:

- *Penicillium rubens* DSM 848 for Xenon Pulsed-Light.

- *Aspergillus carbonarius* DSM 872 with 0.8 mg/g DMSO in the sporulation medium for UV Medium-Pressure lamp.
- *Aspergillus carbonarius* DSM 872 with 1.0 mg/g to 1.4 mg/g DMSO in the sporulation medium for UV Low-Pressure lamp.

These surrogates for Aspergillus species can be used in the future for microbiological validations of UV-based decontamination processes.

IVLV members can download the complete final German project report from our homepage. All you need is to register in the section "My IVLV". Non-members can request the final report from the IVLV office at office@ivlv.org.

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