Bioindicators for UV-radiation - resistance of conidiospores of different *Aspergillus niger* strains

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ABSTRACT

A wide variety of different *A. niger* strains are applied as bioindicators in challenge tests for the quality control of decontamination devices of automated packaging lines. As spores could show a wide variation in the resistance against UV-radiation, the decontamination efficiency values described in the literature are scarcely comparable and there is a need to establish a standardised methodology.

The aim of this study was to determine the resistances of various *Aspergillus niger* strains to UV-radiation and also the influence of the application of different UV-emitting systems.

The investigation of more than 10 strains *A. niger* showed a wide variation of the resistances against UV-radiation. In particular the *A. niger* strains ATCC 8740, DMS 11167 and ATCC 16404 were found to be the most resistant organisms against UV and also low-pressure gas plasma radiation.

As there are official recommendations for the application of deviant *A. niger* strains in challenge tests, the wide variation in the resistance of the different strains shown in this study leads to variable results in the evaluation of decontamination processes, which are scarcely comparable.

In order to allow the comparability of challenge tests a standardisation of challenge test concerning the applied *A. niger* strains could be recommended. To provide highest microbiological safety, an *A. niger* strain with a high UV-radiation resistance could be suggested as a standard bioindicator organism in challenge tests for UV-decontamination devices in packaging and filling lines.

Keywords: food packaging; hygienic filling; UV-radiation; gas plasma; decontamination; *Aspergillus niger*; resistance; challenge test; count reduction test

INTRODUCTION

In view of growing demand for packaged food products with extended shelf-life decontamination of food packaging material is in the focus of packaging material producers and food processors.

Many factors such as pH-value, aw-value, cooling chain and aspired shelf life are influencing the shelf-life and microbiological safety of packaged foods and adequate decontamination processes of the packaging materials are indispensible. From a gentle partial killing of a fraction of vegetative cells (according to a pasteurisation of food) to a complete sterilization, all levels of packaging material decontaminations are applied, using various different technologies [1]. A very common method is the irradiation of the packaging materials with UV-light, especially as application in decontamination devices of automated packaging lines.
To evaluate the efficiency of the decontamination devices in industrial packaging processes, challenge tests are applied, performed as count reduction or end-point-tests [2]. For this purpose the packaging materials are artificially contaminated by test microorganisms as bioindicators. Subsequently the material is processed in the decontamination devices of the filling machines and investigated finally for surviving cells. Log-reduction is calculated as a measure for the efficiency of the decontamination process. Commonly used test microorganisms applied for the evaluation of UV-radiation are conidiospores of Aspergillus niger [3]. Previously studies on the resistance of bacterial endospores of different Bacillus subtilis strains showed significant differences against UV-radiation [4]. Many studies in the literature do not identify the exact strain of A. niger used [5] and also often different strains are suggested as bioindicators [3]. Therefore the decontamination efficiency values described in the literature are scarcely comparable and there is a need to establish a standardised methodology. Especially the resistance of the used organisms should be defined to obtain comparable results.

The aim of this study was to determine the resistances of various Aspergillus niger strains to UV-radiation and also the influence of the application of different UV-emitting systems.

MATERIALS & METHODS

Cultivation of microorganisms
Cultivation of the moulds and the production of the spores was done equally for all strains according to a standard procedure: From a stock agar culture an aliquot is transferred with an inoculating loop into 4 mL sterile Ringer solution and 0.1 mL of this suspension is spread onto an YGC-Agar surface. After 10 days at 30°C the spores are harvested by distributing 3g of sterile sea-sand onto the surface of the mould culture. The mixture from spores and sand are taken from the petri dish, and transferred into sterile Ringer solution. After an ultra sonic treatment, the supernatant, containing the spores is pipetted into a bottle and stored at 4°C.

Count reduction tests
In order to do the irradiation trials, the spores were diluted and the suspension is sprayed onto a glass surface by a two way nozzle. This spraying method enables a very evenly fine distribution of the spores on the surface, avoiding conglomerates and subsequently shadowing of spores. This shadowing would lead to bad results suggesting a too high resistance of the spores. After drying the bioindicators are ready for use to be irradiated by the UV-lamps.

UV-emitting systems
As UV-emitting systems three different devices were applied:
   a) Amalgam Low-pressure lamp (UV-Systec, Bucha, Germany)
      This device emits monochromatic UV light at 254 nm.
   b) Mercury middle-pressure lamp (Dr. Höhnle AG, Gräfelfing, Germany)
      This radiator emits a polychromatic spectrum
   c) Low-pressure Argon plasma (self construction IVV, Freising, Germany)
There is consciously no information given about the absolute irradiation intensity or doses, because this data are hardly measured reliably and the data delivered by the manufacturer of the lamps are not comparable to each other.

\[ D = \frac{\log a - \log b}{t} \]

\( t \) = time of treatment [s]; \( a \) = number of colony forming units at the beginning; \( b \) = number of colony forming units at the end of treatment

**RESULTS & DISCUSSION**

Resistance of more than 10 different *A. niger* strains (among them ATCC 16404 (new nomenclature: *A. brasiliensis*); ATCC 8740; ATCC 6275; ATCC 9642 (new nomenclature: *A. brasiliensis*); ATCC 9029; ATCC 9142; ATCC 32656; ATCC 10577; DSM 11167; DSM 12634 against UV-irradiation was investigated using low-pressure and medium-pressure UV-emitting sources and a low-pressure argon plasma, respectively. Based on previously performed experiments, the distance of the UV-emitting source was chosen with 5 cm distance to sample surfaces. In case of plasma radiation the samples were positioned in the plasma lumen itself. Growing conditions as well as storage atmosphere were kept constant in order to obtain comparable results.

*Low-pressure mercury lamp*

We found a wide range of resistances of the diverse strains. The D-values varied by the factor of 61.1. Highest D-value was found for ATCC 8740, followed with by a factor of 10 lower D-values of DMS 11167 and ATCC 16404. The other strains investigated were found with D-values in the range of 1.9 to 3.7 seconds, among them ATCC 6275.

**Table 1.** Decimal Reduction time (D-value) of different *A. niger* strains affected by low-pressure UV-radiation

<table>
<thead>
<tr>
<th>ATCC</th>
<th>D-value [s]</th>
</tr>
</thead>
<tbody>
<tr>
<td>8740</td>
<td>116.1</td>
</tr>
<tr>
<td>16404*</td>
<td>7.7</td>
</tr>
<tr>
<td>6275</td>
<td>2.4</td>
</tr>
</tbody>
</table>

*new nomenclature: *A. brasiliensis*
Figure 1. Inactivation kinetics of different *Aspergillus niger* strains affected by low-pressure UV-radiation, expressed as colony forming units as a function of time [6].

**Middle-pressure mercury lamp**

In view of the higher radiation intensity the D-values for the treatment of the *A. niger* strains with middle-pressure mercury lamp are found in the range of 0,14 to 1,22 seconds, by a factor of more than 100 lower than by the irradiation via the low-pressure UV-source. The values varied by a factor of 8,7, whereas the ranking in regard to the resistance is nearly the same as using the low-pressure mercury lamp. Highest D-value was found again for ATCC 8740, followed by DMS 11167 and ATCC 16404 showing approximately by a factor of two lower D-values. The ranking of the remaining strains showing D-values between 0,14 and 0,35 seconds was partly deviant in comparison to the results obtained by the low-pressure mercury lamp due to the very similar d-values and the normal microbiological deviations.

**Low-pressure Argon plasma**

The same strains were investigated for their resistance against low-pressure plasma using argon or different argon gas mixtures as plasma source. Here again the strains ATCC 8740, DMS 11167 and ATCC 16404 showed the highest resistance in all cases, followed by the other seven strains with a slightly deviant ranking between the different plasma matrix applied.
In summary the results for all UV-emitting sources show a wide variation of the resistances of the investigated microorganisms. The applied radiation sources are significant influencing reduction times according to their individual radiation intensity, but are not affecting individual resistances of the investigated A. niger strains. ATCC 8740, DMS 11167 and DMS 1988 are found to be the most resistant organisms against UV and also low-pressure gas plasma radiation.

**CONCLUSION**

In view of the fact that different A. niger strains are recommended in the literature and also via official recommendations (e.g. VDMA) for application in challenge tests, the wide variation in the resistance of the different strains shown in this study leads to deviant results in the evaluation of decontamination processes. The comparison of studies carried out using different A. niger strains with large differences in their resistance would lead to unreliable conclusions.

Therefore there is a need of a standardization of challenge tests especially concerning the application of A. niger strains with a defined resistance against UV-radiation to provide a reliable methodology to control the UV-based decontamination of packaging materials for food.

Based on the knowledge of these results many of the existing literature studies could be evaluated in a new manner.

In order to simplify the comparability of challenge tests and to provide highest microbiological safety, an A. niger strain of high UV-radiation resistance could be suggested as a standard bioindicator organism in challenge tests for UV-decontamination devices in packaging and filling lines. ATCC 8740 with its outstanding UV-radiation resistance is not an adequate bioindicator organism, because this strain seems to be too resistant for common decontamination processes. The deactivation would be nearly not measurable during an industrial UV-radiation process.

**REFERENCES**


[3] VDMA-Documents: Hygienic Filling Machines of VDMA Class IV for Liquid and Viscous Foods; Minimum requirements and basic conditions for operation in accordance with specification. 2005; Food Processing and Packaging Machinery Association, Frankfurt, Germany (www.vdma.org)

