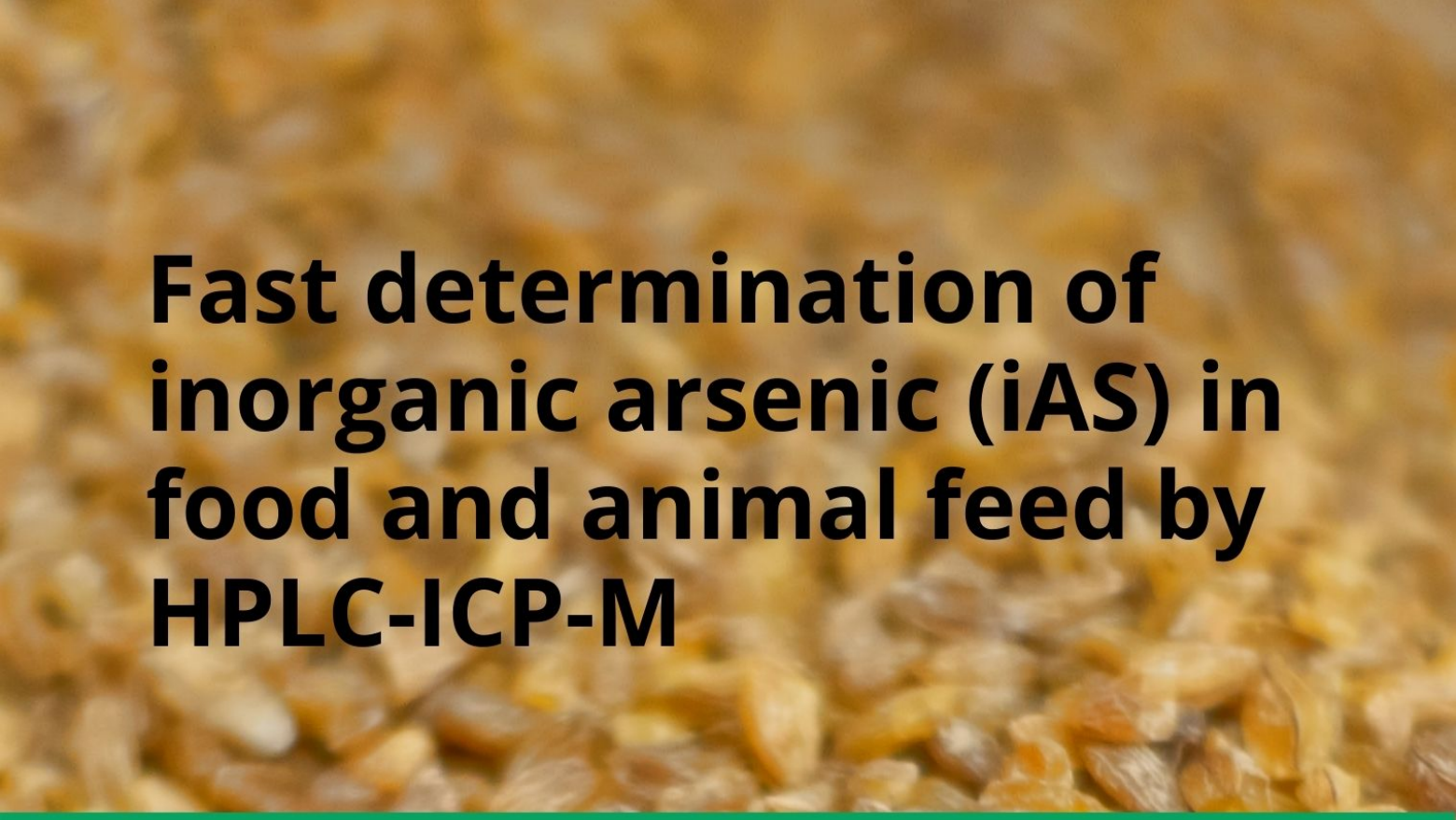

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Spore inactivation on solid surfaces by vaporized hydrogen peroxide—Influence of carrier material surface properties

Elisabeth Eschlbeck, Christina Seeburger, and Ulrich Kulozik

Abstract: The aim of this study was to investigate the influence of surface hydrophobicity and roughness of carrier materials on the inactivation of bacterial spores with gaseous hydrogen peroxide whereas condensate formation is prevented. Spores of *Bacillus subtilis* and *Bacillus atrophaeus* were applied either as single spore culture or as a mixed spore population to simulate natural contamination with microorganisms of different characteristics. Inactivation with gaseous hydrogen peroxide was carried out at 5200 ppm hydrogen peroxide without condensate formation. The inactivation results of *B. subtilis* and *B. atrophaeus* spores on carrier materials with varying surface hydrophobicity differed significantly. However, inactivation of the mixed spore populations resulted in similar resistance compared to the single spore batches. The results of this study indicate that surface hydrophobicity most probably has an impact on the inactivation with gaseous hydrogen peroxide whereas surface roughness only plays a minor role.

Keywords: bacterial spores, disinfection, gaseous hydrogen peroxide, hydrophobicity, packaging

1. INTRODUCTION

For aseptic packaging processes, hydrogen peroxide (H_2O_2) in its vaporized form is an often applied decontamination agent. Many of the influencing factors, such as temperature, concentration, amount of water in the gas, have been reported elsewhere (Pruß, Stirtzel, & Kulozik, 2012; Unger-Bimczok, Kottke, Hertel, & Rauschnabel, 2008; Wang & Toledo, 1986). The influence of surface hydrophobicity of the carrier material (CM) and the bacterial spores, respectively, during a process with condensate formation has also been studied (Eschlbeck, Seeburger, & Kulozik, 2018). However, little is known about the influence of surface hydrophobicity and surface roughness on the inactivation by vaporized H_2O_2 without condensate formation. In case of absence of condensate formation, this process can be seen as a dry process. However, even in a dry process, an effect of the surface properties could be expected and help to explain often unexpected and variable inactivation results.

When vaporized H_2O_2 is applied, the formation of condensate depends on the amount of water and H_2O_2 in the gas as well as on the saturation pressure and temperature (Beysens, 1995). According to Parks and Watling (2004), the dew point temperature can be calculated as a function of constant saturation pressure and gas concentration. The dew point temperature is the temperature below which condensate begins to form. If the temperature of the gas is constantly above the dew point temperature, the process is not affected by condensate formation. This includes the gas close to the surface. Therefore, the surface temperature of all surfaces in contact with the gas has to be above the dew point temperature as well to prevent condensate formation.

However, a certain reversible occupancy of the surface with gas molecules takes place which is called adsorption (Dörfler, 2002). Adsorption is influenced by the surface hydrophobicity and polarity of the adsorbent on the one hand and on the polarity of the gas molecules on the other hand. The degree of polarity of a single molecule is indicated by its dipole moment. Water molecules have a dipole moment of 1.84 Debye (Law, 2014; Mortimer & Müller, 2010), whereas H_2O_2 molecules have a dipole moment of 2.21 Debye (Yu and Yang 2011). As the dipole moment of H_2O_2 is higher, the adsorption on hydrophilic surfaces might be enhanced compared to more hydrophobic surfaces. Besides this, the overall adsorption of H_2O_2 and water is higher on hydrophilic carrier material compared to hydrophobic material. Therefore, on hydrophilic CMs, inactivation of bacterial spores is expected to be less effective as the H_2O_2 molecules adsorb on the surface are not present in the gas anymore and, thus, cannot inactivate microorganisms. At the same time, microorganisms, which are more hydrophilic than the CM would be preferred adsorption spots and, thus, inactivation would be enhanced.

At the same time, high surface roughness leads to a larger surface on which adsorption can take place. In addition, surface roughness might induce microcondensation resulting in locally variable, higher or lower H_2O_2 concentration. Besides, surface roughness provides the chance for microorganisms to be covered and therefore be protected from the inactivation effect (Unger et al., 2007).

The German VDMA, "Verband Deutscher Maschinen- und Anlagenbau e. V." (2002), recommends spores of *Bacillus subtilis* (*B. subtilis*) or *Bacillus atrophaeus* (*B. atrophaeus*) as test cultures or "bioindicators" (BIs) to monitor H_2O_2 inactivation processes. However, no CMs are recommended. Commercially available BIs consist of one spore species, which is either already applied on a CM, for example, a paper strip dispersed in water or ethanol. The BIs are brought into the decontamination device or filling machine and subsequently inactivated in a sterilization test run. Together with a reference BI, which is not treated, the inactivation effect can be assessed. However, surface contamination in reality displays a mix of different microorganisms with different surface characteristics. Assuming that surface hydrophobicity of the CM influences the adsorption of vaporized H_2O_2, surface

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hydrophobicity of bacterial spores should have an impact on the inactivation results as well.

The mechanism of bacterial spore inactivation with H_2O_2 gas is quite complex and not yet fully understood. Finnegan et al. (2010) investigated the inactivation with gaseous H_2O_2 compared to liquid H_2O_2 and concluded that the gas irrupts deeper into the spore structure than liquid H_2O_2 and most probably oxidizes amino acids essential for the spore germination.

Pruß et al. (2012) investigated the influence of different surface temperatures on the inactivation with gaseous H_2O_2. The authors performed inactivation experiments with the same gas concentration and temperature, while varying the surface temperature of the CM. This resulted in a dry process for a high CM temperature and condensate formation for a low CM temperature.

Sigwarth and Stärk (2003) applied different CMs to investigate their influence on the resistance of *Geobacillus stearothermophilus* (*G. stearothermophilus*) spores against gaseous H_2O_2. Their results show that surface roughness influences the inactivation results. Extremely rough surfaces increase bacterial spore resistance. However, no clear correlation could be found between surface roughness and spore resistance.

Rogers et al. (2005) studied the inactivation of spores of *Bacillus anthracis*, *B. subtilis*, and *G. stearothermophilus* with gaseous H_2O_2 on different surfaces. The authors concluded that—due to porosities and cavities of the surfaces—spores might be sheltered and, thus, show higher resistance on those materials. However, the conditions applied by these authors are far from real conditions of packaging material properties.

The influence of different CMs was also examined by Unger et al. (2007). They applied G. *stearothermophilus* spores to evaluate the influence of roughness during a clean room decontamination cycle. However, they applied relatively low amounts of H_2O_2 for a long time. No correlation could be observed. The authors, however, assume that there is some kind of relationship between roughness and resistance of spores against gaseous H_2O_2.

Grand et al. (2010) investigated the influence of different CMs. However, they applied two different methods for CM contamination which are both not suitable to generate a monolayer of bacterial spores. Therefore, spores can be protected by several layers in spore clusters or cavities and thus show different inactivation kinetics compared to spore monolayers. A final conclusion about the sole influence of surface hydrophobicity or roughness of the CM from their results is thus not possible.

Recent literature indicates that there might be an influence of CM roughness and surface hydrophobicity on the inactivation with gaseous H_2O_2. However, those characteristics have not been sufficiently investigated yet.

The purpose of this study, therefore, is to investigate the influence of surface roughness and hydrophobicity on the inactivation of bacterial spores with gaseous, noncondensing hydrogen peroxide.

2. MATERIALS AND METHODS

2.1 Bacterial spores

Bacillus atrophaeus (DSM 675) and *B. subtilis* (DSM 4181) were obtained from the DSMZ (German collection of microorganism and cell cultures, Braunschweig, DE). Both spore formers were cultivated with sporulation media (for 1,000 mL of distilled water: 5.0 g peptone from casein (Gerbu, Heidelberg, DE), 3.0 g beef extract (Gerbu), 3.5 g potassium chloride (Merck, Darmstadt, DE), 250 mg magnesium sulfate (Roth, Karlsruhe, DE), after autoclaving 10 mL of 10% glucose in water (Merck) and 1 mL of the following micronutrients were added: 1 M calcium nitrate (Sigma Aldrich, Darmstadt, DE), 0.01 M manganese chloride (Merck), and 1 mM iron sulfate (Fluka, Seelze, DE). For spore production of *B. subtilis,* the microorganisms were cultivated in a bioreactor (Eschlbeck, Bauer, and Kulozik (2017), *B. atrophaeus* was cultivated on agar plates. The addition of agar-agar (15 g/L, Fisher Scientific, Schwerte, DE) resulted in solid sporulation media.

After 10 days at 37 °C, *B. atrophaeus* spores were removed from agar plates by means of 10 mL sterile distilled water, suspending the spores with a spatula and collecting the suspension. For *B. subtilis* spores, a static cultivation pH of 8.5, temperature of 37 °C, and constant oxygen supply of 2 L/min of sterile filtered air were applied. Nonenzymatic purification of both spore species was achieved by heat activation (80 °C, 20 min) and at least four subsequent washing steps with sterile, distilled water, followed by centrifugation at 4,000 *g* for 10 min at 4 °C. This step was necessary to remove vegetative cells and cell debris. The purity of the spores was verified with a light microscope (Axioskop, Carl Zeiss, Oberkochen, DE) where spores appear brighter than vegetative bacteria due to their contained complex consisting of dipicolinic acid (pyridine-2,6-dicarboxylic acid) and calcium (Antranikian, 2006). Washing steps were repeated until a purity of the BI obtained was at least 95% spores. Both spores were stored in distilled water at 4 °C.

2.2 Contact angle measurement

This method to quantify surface hydrophobicity was carried out as described by Eschlbeck and Kulozik (2017). Spores were filtered on a cellulose acetate filter (pore size: 0.22 µm, Sartorius Stedim, Göttingen, DE), a subsequent equilibration time of 120 min on 2% agar-agar (Fisher Scientific) was applied. The filters with spores were air dried for 50 min. On applying the sessile drop method, a drop of 8 µL Milli-Q water was set on the filter surface during the plateau time, and a video of the first 6 s was taken. The resulting pictures were analyzed with DSA 4 (Krüss, Hamburg, DE), pictures taken between 2 and 4 s after the onset of drop deposition showed consistent water contact angles and were therefore used. Three independent filters were prepared for each spore suspension whereas a minimum of five drops was deposited and evaluated on every filter.

2.3 Carrier materials

Water contact angle measurement of four different CMs was carried out as described above. The results were already published in Eschlbeck et al. (2018). The chosen materials and the respective surface hydrophobicity are depicted in Table 1.

Pieces of cardboard packaging material pieces (surface: low density polyethylene (LDPE), size: 40 × 55 mm, provided by Tetra Pak, Modena, Italy) were the basic material. Thin films of the different CMs were mounted on this LDPE cardboard material with double-sided adhesive tape. The basic material was sterilized with gamma radiation (25 kGy, Synergy Health, Allershausen, DE). The mounted thin films were sterilized by means of gaseous hydrogen peroxide (9,000 ppm, 20 s, 70 °C) with the hydrogen peroxide decontamination device.

2.4 Surface roughness

Surface roughness was investigated by means of an atomic force microscope (Alpha-500, WITec, Ulm, DE) at the 'Fraunhofer Institut für Verfahrenstechnik und Verpackung' (IVV) (Freising, DE). Dry CMs were cut in small pieces and fixed on specimen

Table 1–Carrier materials and water contact angles (Eschlbeck et al., 2018).

Abbreviation	Materials	Water contact angle	Standard deviation
CM 1	LDPE cardboard packaging material (Tetra Pak)	115°	2.08°
CM 2	PET film, optically rough (23 µm thick, Huthamaki, Ronsberg, DE)	101°	2.69°
CM 3	PET film, optically smooth (23 µm thick, Huthamaki)	81°	1.40°
CM 4	PET film covered with silicium oxide (23 µm thick, Huthamaki)	30°	0.64°

holders by means of double-sided adhesive tape. Therefore, concavities in the test materials were avoided. The size of the examined surfaces was 50 × 50 µm for CM 1, CM 3, and CM 4 and 50 × 100 µm for CM 2. For each CM, three independent spots were measured and the average surface roughness R_z was calculated (Eq. 1).

$$R_z = \frac{1}{3}\sum_{i=1}^{3} R_z(i) \quad (1)$$

$R_z(i)$ is the distance between the highest and lowest detected peaks of the respective sample. In this manuscript, the difference between those peaks specifies the surface roughness.

2.5 Application of biological indicators to the test material surface

A total of 300 µL of spore suspension was mixed with 700 µL ethanol (Merck), 10 µL of the resulting mixture with approximately 108 spores/mL was spread on CMs with a spatula (described by Pruß et al., 2012). Each CM held an amount of 10^6 spores.

A mixture of both spore species was generated (150 µL of each spore suspension, same concentration as described before) for the experiments with a mixed spore population. BIs were dried under sterile conditions for at least 1 hr. Electron microscopic pictures were taken to verify even distribution of the spores, data not shown.

2.6 Hydrogen peroxide treatment

Liquid hydrogen peroxide (35%, 11.63 mol/L, Evonik Industries, Essen, DE) was completely evaporated (Controlled Evaporator Mixer, Bronkhorst, NL). Temperature of the treatment chamber was set to 70 °C. Vapor temperature was constantly measured with two temperature sensors (Angled Thermocouple, Pentronic, Sweden) that were integrated into the treatment chamber. The catalytic material of one of the sensors dissolves hydrogen peroxide in an exothermic reaction (Pruss (2013), the resulting temperature difference correlates with the concentration of H_2O_2 in the gas. For all experiments, H_2O_2 concentration was set to 5200 ppm and continuously monitored (LabVIEW, National Instruments, UT, USA).

BIs were inserted into the treatment chamber for the respective treatment time (0, 2, 4, 6, 8, or 10 s). Directly after the treatment, BIs were instantaneously removed and immediately covered with 10 mL of a tween-catalase solution (9.9 mL sterile, 0.1% tween 80 [Gerbu Biotechnik GmbH], 0.1 mL of catalase from *Micrococcus lysodeikticus* [Sigma Aldrich]) to keep the time between the end of the experiment and the beginning of sample preparation for analysis to a minimum (about 0.5 to 1 s). Therefore, potentially remaining hydrogen peroxide was immediately dissolved by catalase, preventing any residual H_2O_2 activity. Spores were detached from the CM by magnetic stirring (200 rpm, Arex Digital Heating Magnetic Stirrer, VELP Scientifica, Usmate, ITA), dilution series were created and surviving spores were detected on plate count agar (as described above). Incubation parameters were 30 °C for 48 hr. Due to the fact that *B. subtilis* spores form white, opal colonies whereas *B. atrophaeus* colonies show smaller, glossy yellow appearance, surviving spores can be distinguished and individual inactivation kinetics can be generated for *B. subtilis* and *B. atrophaeus*.

Inactivation experiments were carried out for each CM with *B. subtilis* spores, *B. atrophaeus* spores, and the mixed spore population. All experiments were carried out in triplicate.

The dew point temperature of gaseous H_2O_2 was calculated according to Parks and Watling (2004) and was 43.6 °C. To ensure an inactivation process without condensate, the initial surface temperature of each CMs was raised to 65 °C (Arex Digital Heating Magnetic Stirrer, VELP Scientifica).

During the treatment, surface temperature of all CMs was monitored with an infrared thermometer (FLIR i3, FLIR Systems GmbH, Frankfurt am Main, DE). Surface temperature of the CM did not vary significantly during 10 s of treatment time.

Results are presented as logarithmic survival rate as calculated and applied for the graphical presentation. The survival rate (log $S(t)$) is calculated by dividing the amount of microorganisms (N) surviving at a time t by the initial number of microorganisms (N_0) (Eq. 2).

$$\log_{10} S(t) = \frac{N}{N_0} \quad (2)$$

2.7 Statistical methods

All experiments were carried out in triplicate; the standard deviation was calculated and is depicted in the figures. A t-test for independent samples with a level of significance (α) of 0.05 was applied to assess if results differ significantly.

3. RESULTS AND DISCUSSION

3.1 Surface hydrophobicity of CMs

Water contact angle data of the applied CMs and bacterial spores were already published in Eschlbeck et al. (2018). For a better understanding of the influence of surface hydrophobicity on the inactivation with gaseous H_2O_2, the results are depicted in Figure 1.

Both materials CM 1 and CM 2 are more hydrophobic than the spores of *B. subtilis*. Surface hydrophobicity of CM 3 is between the two bacterial spores whereas CM 4 is more hydrophilic than both spore formers and with a water contact angle of 30°, the most hydrophilic CM under investigation. Surface hydrophobicity of spores of *B. subtilis* compared to spores of *B. atrophaeus* differs significantly.

3.2 Surface roughness of CMs

The resulting average surface roughness values are displayed in Table 2. Clearly, CM 2 has the highest roughness whereas CM 1 is smoother and CM 3 exhibits only little roughness.

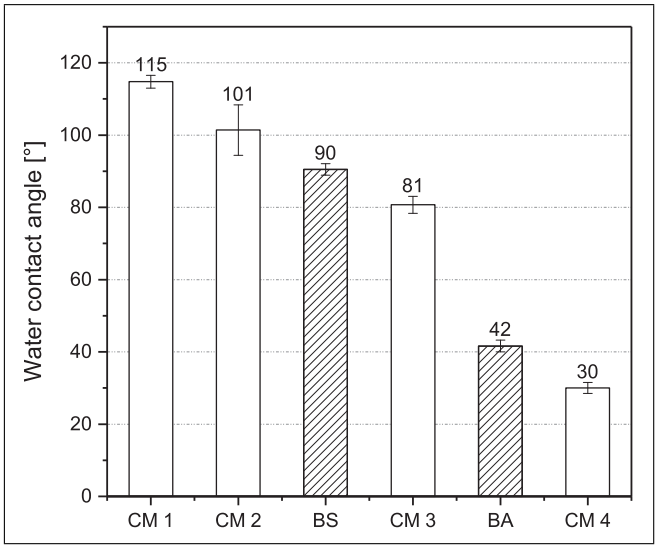

Figure 1–Water contact angles of the carrier materials (CM 1 to CM 4) and *B. subtilis* (BS) as well as *B. atrophaeus* (BA) spores (Eschlbeck et al., 2018).

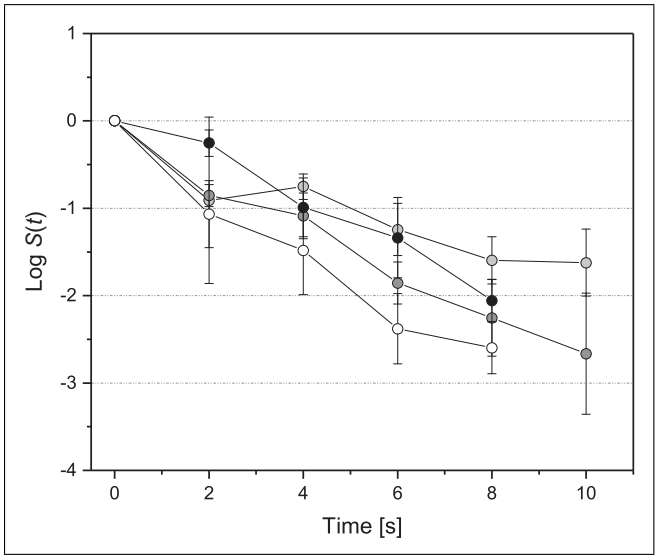

Figure 2–Inactivation results of *B. subtilis* spores on CM 1 (black), CM 2 (dark gray), CM 3 (bright gray), and CM 4 (white) with gaseous H_2O_2 (5200 ppm, 70 °C).

Table 2–Average surface roughness R_z of the different carrier materials.

	CM 1	CM 2	CM 3	CM 4
R_z (nm±)	3800 (±477)	16038 (±895)	304 (±22)	Not measurable

However, atomic force microscopy (AFM) of CM 4 was not possible as the cantilever of the AFM could not identify any roughness of this surface. We expect CM 4 to be having the most smoother surface as Vasko (2006) also examined the surface roughness of silicium oxide with an arithmetic mean roughness R_a of 0.86 nm.

3.3 Impact of different CMs on the spore recovery rate

The spore recovery data of the untreated samples (treated for 0 s) did not differ significantly from the amount of spores applied originally. Therefore, neither surface hydrophobicity nor surface roughness of the CM has a significant influence on the spore recovery in the range investigated in this study.

3.4 Impact of different CMs on the inactivation effect

The inactivation kinetics of *B. subtilis* spores on different CMs are depicted in Figure 2. After the first 2 s, the inactivation kinetics can be seen as log-linear with CM 3 as CM which provides the best survival and CM 4 as CM with the fastest inactivation. CM 1 and CM 2 show an inactivation of approximately 2 log after 8 s.

The inactivation of spores of *B. subtilis* seems to be independent of the surface hydrophobicity of the CMs as the slowest inactivation and, thus, highest resistance results from the combination with CM 3. CM 3 has a similar water contact angle (81°) as the spores of *B. subtilis* (90°). The inactivation results on CM 1 and CM 2, which are more hydrophobic, are similar and show a lower resistance than CM 3. CM 4, which is most hydrophilic, leads to the fastest inactivation of *B. subtilis* spores.

The inactivation kinetics of *B. atrophaeus* spores are displayed in Figure 3. Except for CM 4, the spores of *B. atrophaeus* are less resistant on all CMs against dry gaseous H_2O_2 than the spores of *B. subtilis*. Less resistant means that inactivation of *B. atrophaeus* spores is faster when inactivated under the same conditions than

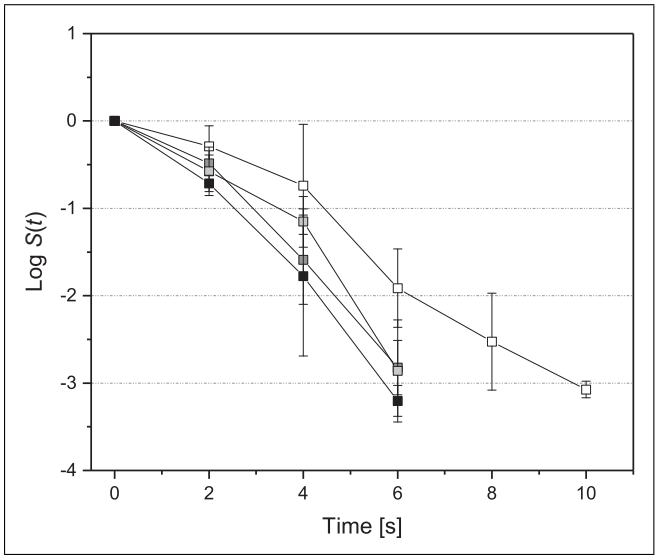

Figure 3–Inactivation results of *B. atrophaeus* spores on CM 1 (black), CM 2 (dark gray), CM 3 (bright gray), and CM 4 (white) with gaseous H_2O_2 (5200 ppm, 70 °C).

B. subtilis spores. After a treatment time of 6 s, almost 3 log of inactivation was achieved on CM 1, CM 2, and CM 3. Inactivation of the spores on the most hydrophilic CM, CM 4, results in an inactivation time of 10 s for 3 log. An inactivation time of 6 s leads to significantly better survival of the spores applied to CM 4 compared to the other CMs. This is contrary to the inactivation of *B. subtilis* spores (Figure 2) as they are less resistant on CM 4.

Inactivation with gaseous H_2O_2 compared to liquid H_2O_2 was investigated by Finnegan et al. (2010). The authors suppose that due to the higher kinetic energy of the gaseous H_2O_2, molecules erupt deeper into the spore structure and inactivate the spore by oxidation of inner structures. If inner spore structures are responsible for spore resistance against gaseous H_2O_2, it is supposedly their hydrophobic characteristic that leads to more or less adsorption of the gas molecules. To the best of our knowledge,

literature does not provide information on how to measure the inner surface characteristics. Water contact angle measurement only depicts the surface characteristics of the outermost spore layer, which might be one explanation why no correlation could be observed.

Assuming that H_2O_2 gas molecules adsorb on the most hydrophilic surface available, the H_2O_2 molecules would adsorb on CM 4 and, therefore, be not available in the gas anymore. Therefore, the inactivation is expected to be less pronounced. For *B. atrophaeus*, this effect can be observed, but not for *B. subtilis* spores.

Comparing the inactivation kinetics on CM 4 of the two spore species, their kinetics are similar. For both bacterial spores, inactivation of 3 log occurred after 8 s of treatment time. Therefore, we suppose that adsorption on hydrophilic CM 4 results in uniformity of the inactivation. On the more hydrophobic surfaces, less molecules adsorb on the surface and inner spore structures and specific spore resistance to gaseous H_2O_2 are decisive for spore survival.

One possible explanation consists in the abrupt ending of the decontamination. This leads to a concentration gradient between the environment without H_2O_2 and the surface with adsorbed H_2O_2 molecule. By means of this gradient, molecules desorb and for a very short time, a high concentration of H_2O_2 molecules close to the surface develops. This might lead to similar inactivation effects of spores on hydrophilic surfaces.

3.5 Influence of surface roughness

Interestingly, the surface roughness only seems to play a minor role, at least in the range investigated in this study. For *B. subtilis* spores as single spore species (Figure 2), the inactivation on smooth CM 3 results in the highest spore resistance. The fastest inactivation takes place with CM 4, which is assumed to be even smoother. Besides, the highest average surface roughness of 16 µm might not provide enough space for microorganisms to hide or be sheltered from the inactivating gas.

3.6 Effect of gaseous H_2O_2 on a mixed spore population

A comparison of the inactivation kinetics of the single spore batches and the mixed spore cultures (Figure 4 to 7) reveals that within the standard deviations, there are no significant differences in the inactivation rate.

In Figure 7, the inactivation kinetics of both spore species on CM 4 are displayed. Their inactivation results as single spore species are quite similar with an inactivation of 2.5 log after 8 s of treatment. If there is an intense adsorption of H_2O_2 molecules on CM 4, the molecules adsorb also on the surface of the CM, which is very close to the spores. As adsorption is a reversible process, the desorption during the short time between removing of the BI from the decontamination device and decomposition of adsorbing H_2O_2 molecules, which takes approximately 1 s might increase the concentration of H_2O_2 in close surrounding of the spores, and lead to a similar inactivation of both spore species.

If there was an influence of spore surface hydrophobicity, the inactivation of *B. subtilis* spores would be less intense in a mixed spore population as the H_2O_2 molecules would prefer the more hydrophilic *B. atrophaeus* spores for adsorption. Due to the fact that there is no significant difference in the inactivation of single spore species and mixed spore populations, we conclude that the surface hydrophobicity of bacterial spores measured by water contact angle measurement does not influence the inactivation with dry gaseous H_2O_2.

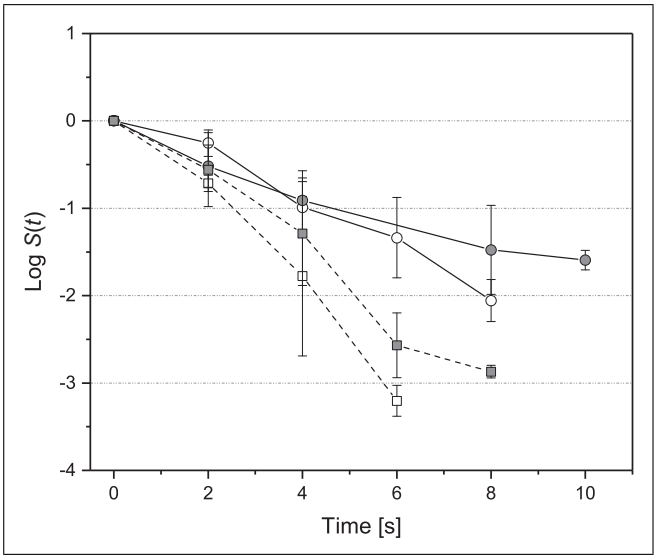

Figure 4–Inactivation with gaseous H_2O_2 (5200 ppm, 70 °C) on CM 1. White circle: *B. subtilis* spores single culture, gray circle: *B. subtilis* spores mixed culture, white square: *B. atrophaeus* spores single culture, gray square: *B. atrophaeus* spores mixed culture.

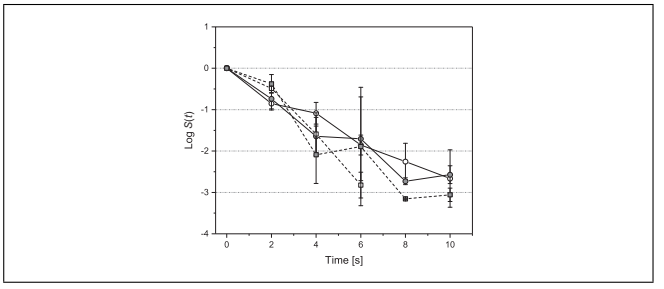

Figure 5–Inactivation with gaseous H_2O_2 (5200 ppm, 70 °C) on CM 2. White circle: *B. subtilis* spores single culture, gray circle: *B. subtilis* spores mixed culture, white square: *B. atrophaeus* spores single culture, gray square: *B. atrophaeus* spores mixed culture.

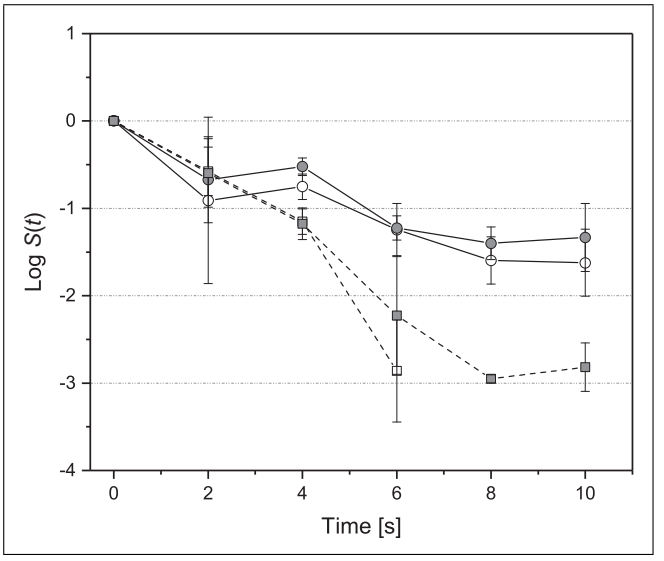

Figure 6–Inactivation with gaseous H_2O_2 (5200 ppm, 70 °C) on CM 3. White circle: *B. subtilis* spores single culture, gray circle: *B. subtilis* spores mixed culture, white square: *B. atrophaeus* spores single culture, gray square: *B. atrophaeus* spores mixed culture.

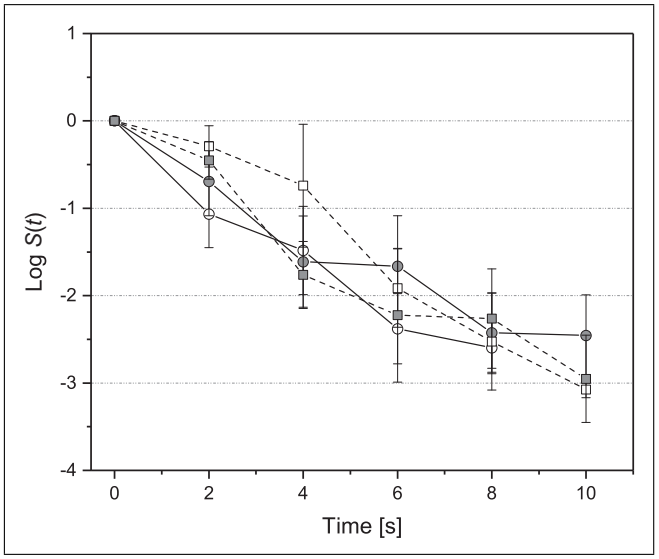

Figure 7—Inactivation with gaseous H_2O_2 (5200 ppm, 70 °C) on CM 4. White circle: *B. subtilis* spores single culture, gray circle: *B. subtilis* spores mixed culture, white square: *B. atrophaeus* spores single culture, gray square: *B. atrophaeus* spores mixed culture.

Our results are in accordance with the results obtained by Unger et al. (2007) although the applied H_2O_2 concentration of this study, the resulting dew point temperature, and the CM temperature to prevent condensate formation are much higher.

4. CONCLUSION

Concluding, surface hydrophobicity of the CM does most probably have an impact on inactivation during a dry H_2O_2 gas process. However, the mode of action of gaseous spore inactivation is complex and not yet fully understood. Our results indicate that it is essential to consider the specific characteristic of the surface when it comes to the layout of sterilization cycles of clean rooms or production of BI as there is an influence of the surface even during a dry H_2O_2 process. To obtain meaningful inactivation validation test results, the surface material of interest has to be applied as CM for the BI.

Further experiments with a broad range of CMs of different surface hydrophobicity and roughness are required to elucidate systematic relationships between the inactivation effect and the surface characteristics during a dry gaseous H_2O_2 process.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

The bacterial spores were cultivated and purified by E. Eschlbeck. She planned and supervised the experiments with support and critical discussions from U. Kulozik, who had also initiated the study. E. Eschlbeck and C. Seeburger conducted the contact angle measurements, prepared the materials for the AFM, executed the AFM, conducted the inactivation experiments, and discussed the results. Analysis of the data, interpretation of datasets and plotting of data were done by E. Eschlbeck and C. Seeburger with supervision of U. Kulozik. The manuscript was essentially written by E. Eschlbeck; C. Seeburger and U. Kulozik contributed to the manuscript by critical reading and several discussions.

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