Original Article

Newly developed resistometer to determine the resistance of biological indicators for monitoring VH_2O_2 sterilization processes depending on temperature, water vapor and hydrogen peroxide gas concentrations

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Abstract

Background: Vaporized hydrogen peroxide/water vapor (VH_2O_2/VH_2O) is used for the sterilization of heat-sensitive medical devices as well as for the disinfection of rooms and ambulance vehicles. The reaction kinetics of spore inactivation by VH_2O_2 and the influence of temperature and water vapor concentration have not yet been adequately clarified in publications.

Methods: In order to investigate the dependence of the inactivation rate on the individual variables, VH_2O_2, VH_2O concentration ($[VH_2O_2]$, $[VH_2O]$) and temperature, and their parameters, a VH_2O_2/VH_2O-Resistometer has been developed that enables biological and chemical indicators to be inserted into a defined gas flow with adjustable, constant process parameters, so that all critical variables could be tested individually.

Results: With this setup D-values in a range from 1.1 to 10.4 min and a temperature dependent z-value of 43.7 K were measured for *G. stearothermophilus* in various sterilization processes. The reaction kinetics were determined with respect to $[VH_2O_2]$ and $[VH_2O]$, whereby it could be shown that the inactivation of the *G. stearothermophilus* biological indicators corresponds to reaction kinetics of approx. 1.4th order in relation to $[VH_2O_2]$. In addition, the water vapor concentration under non-condensing conditions had a negative influence on the spore inactivation rate if the $[VH_2O_2]$ was low (<780 ppm/1.03 mg/l). At a $[VH_2O_2] \geq 780$ ppm (1.03 mg/l), however, no effect of water vapor concentration could be observed.

Conclusions: The resistometer described here enables further questions about VH_2O_2/VH_2O sterilization processes to be clarified, such as the dependence of the D-value on spore cultivation conditions and carriers. Various information can be obtained, for example for the international standardization work and the general understanding of VH_2O_2/VH_2O sterilization processes.

Keywords
- VH_2O_2 Sterilization
- VH_2O_2 Resistometer
- VH_2O_2 Biological indicator
- VH_2O_2 D-value determination
- VH_2O_2 z-value determination
- VH_2O_2 Reaction kinetics

Introduction

Sterilization with vaporized hydrogen peroxide/water vapor (VH_2O_2/VH_2O) is used in healthcare for reprocessing of reusable and single-use instruments and industrially for sterilization of medical devices which cannot be sterilized at higher temperatures. Furthermore, hydrogen peroxide/water mixtures are evaporated in decontamination processes to disinfect rooms and ambulance vehicles.

Sterilization of medical devices is usually performed using vacuum processes in which H_2O_2/H_2O mixtures are injected and vaporized, while for room and vehicle disinfection, H_2O_2/H_2O solutions are nebulized as aerosols and/or vaporized under ambient pressure.

Despite these widespread healthcare applications of VH_2O_2/VH_2O sterilization processes, there are no DIN,

EN or ISO standards published yet that specifically regulate the validation and routine monitoring of VH_2O_2 sterilization processes or enable the uniform determination of the resistance of biological indicators for monitoring these processes. These standards are currently under development. Until their development is completed and valid, only the EN ISO 14937 and 11138-1 standards can be used, which are generally applicable to all sterilization processes and biological indicators [1, 2].

Problem description

For the validation and monitoring of sterilization and disinfection processes, it is necessary to know the reaction kinetics of germ inactivation of the respective process [1]. In order to evaluate this connection and to ensure that sufficient germs are inactivated, biological indicators with known resistance are required for each sterilization process.

The general biological indicator standard EN ISO 11138-1 [2] provides specific test conditions for the determination of the test germ resistance and refers to the standard EN ISO 18472, which describes specifications for corresponding test sterilizers (resistometers) [3]. The standard requires that all critical variables and their parameters have constant values over time (steady state), "except for the process variable [time], which is used to determine the D-value" [2, Annex D].

As reported [4], this requirement is difficult to implement. For example, in vacuum processes such as those in typical hospital sterilizers, adsorption and reaction of the H_2O_2 on walls, samples, and packaging cause a decrease in $[VH_2O_2]$ and an increase in $[VH_2O]$ over the time.

If the D-value is still calculated on the basis of an average concentration value despite of a decreasing $[VH_2O_2]$, this is only correct provided that the sterilization process kinetics are of first order in relation to the spore population only and are not influenced by changes in the $[VH_2O]$ and $[VH_2O_2]$. The exponents of the concentrations therefore play an essential role in the reaction kinetics, but have not yet been determined.

When the resistance is determined in liquid H_2O_2/H_2O solution, the process variables can be reproducibly kept constant and measured [5]. However, it is questionable if the results obtained in solution can be transferred to sterilization with gaseous H_2O_2/H_2O mixtures, since the mechanisms of action of H_2O_2 in the liquid and gaseous phases are probably different [6,7].

Decontamination systems under normal pressure in isolators ("glovebox") achieve an approximately constant $[VH_2O_2]$ and $[VH_2O]$ by continuously adding evaporated H_2O_2/H_2O solution. However, there is also a possibility of locally or temporally varying $[VH_2O_2]$ and $[VH_2O]$ in these isolators due to condensation and the decomposition of hydrogen peroxide [8–10].

The research group of Prof. Kulozik (TU Munich) has presented a resistometer that has constant $[VH_2O_2]$ and $[VH_2O]$ in the gas phase, allowing EN ISO 11138-1 compliant measurements [11,12]. The analyses there were performed using the test germ *Bacillus atrophaeus*, and the reaction kinetics with respect to $[VH_2O_2]$ and $[VH_2O]$ were not determined.

Studies using the various methods described confirm the dependence of the D-value on the $[H_2O_2]$ and temperature. However, it is not clarified to which extent the above-mentioned variables enter into the reaction kinetics of the germ inactivation.

Furthermore, the influence of the water vapor concentration $[VH_2O]$ on germicidal activity in the VH_2O_2 process is under discussion, as previous studies have provided contradictory results. For example, Chung et al [13] reported an inhibition of germicidal activity with increasing $[VH_2O]$, while Taizo et al [14] and Unger-Bimczok et al [15] described an increasing germicidal effect at higher $[VH_2O]$. In another study, there was no effect of water vapor concentration under certain conditions [10]. It should be noted, however, that condensation increased in some of these studies as $[VH_2O]$ was increased. Condensation in VH_2O_2 decontamination processes apparently leads to faster germ inactivation, since the condensate is deposited directly on the parts to be disinfected. However, VH_2O_2 condensation can hardly be controlled and affects the reproducibility of the processes [9, 16].

For the investigations presented here, a test sterilizer (resistometer) has been developed to perform VH_2O_2/VH_2O sterilization processes under non-condensing, constant conditions. With this resistometer the dependence of spore inactivation on $[VH_2O_2]$, $[VH_2O]$ and temperature has been determined.

Material and methods

Description of the resistometer design

A new VH_2O_2 resistometer has been developed in which biological or chemical indicators can be inserted into or removed from the process chamber in less than one second. This meets the standard requirements of constant $[VH_2O_2]$ and $[VH_2O]$ under true "square wave" conditions [2,3,17].

To avoid local and temporal changes in $[VH_2O_2]$ and $[VH_2O]$ due to adsorption and reaction, a procedure in which the concentrations are kept constant by continuous gas exchange in the process chamber has been used for the resistometer (see Fig. 1). In an inert gas stream, adjustable amounts and concentrations of liquid H_2O_2/H_2O mixtures are continuously injected and evaporated. The gas mixture containing H_2O_2 and H_2O flows uniformly through the test chamber and biological or chemical indicators to be measured are inserted into the flowing gas stream. The indicators are placed in a gas-permeable sample holder, pre-temperature-conditioned and exposed to the gas flow for the desired process time.

The resistometer shown schematically in Fig. 1 consists of a process chamber and a preconditioning chamber, which are connected to each other by a guide rail for the sample holder. Warm, dry air flows through the preconditioning chamber and is used for thermal preconditioning of the samples. This will prevent condensation of H_2O_2 on cold sample surfaces in the process chamber which could lead to falsified results.

The carrier gas/VH_2O_2/VH_2O mixture, which is generated in an evaporator, flows continuously through the process chamber. Since this is an open system, there is almost ambient pressure in the process chamber. The carrier gas used in the experiments presented here is dried compressed air, which is regulated to the desired flow rate by means of an adjustable valve for each chamber. To ensure sufficient gas exchange and therefore a constant $[VH_2O_2]$ and $[VH_2O]$ in the area of the samples, the required gas volume flow was determined in preliminary

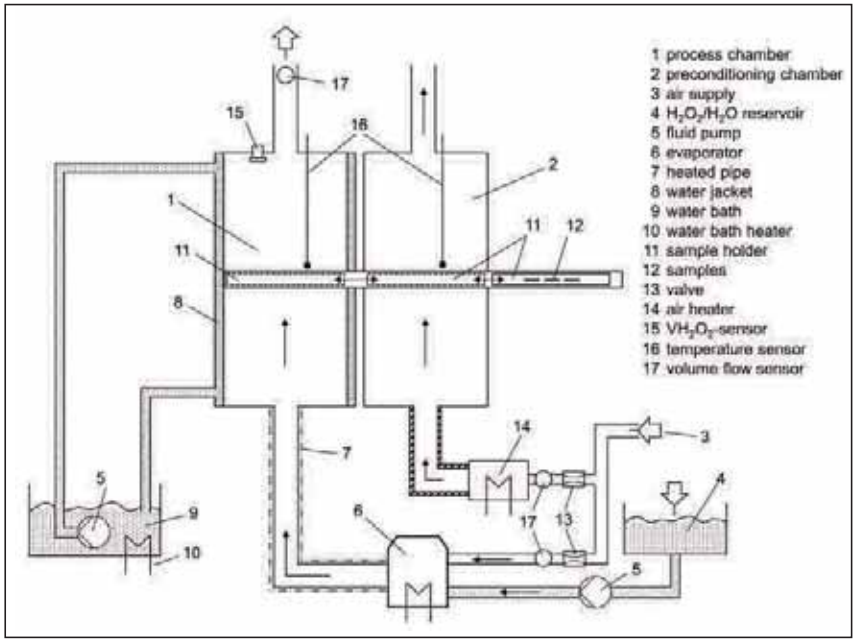

Fig. 1: Schematic setup of the VH_2O_2 resistometer. Explanation in the text.

30 °C

	195 ppm 0.26 mg/l	390 ppm 0.52 mg/l	585 ppm 0.78 mg/l	780 ppm 1.03 mg/l VH_2O_2
740 ppm 0.52 mg/l VH_2O	34%	50%	60%	n.A.*
1460 ppm 1.03 mg/l VH_2O	20%	34%	43%	50%
2950 ppm 2.06 mg/l VH_2O	11%	20%	27,3%	34%
4400 ppm 3.09 mg/l VH_2O	n.A.*	14,3%	20%	25%

40 °C

	195 ppm 0.26 mg/l	390 ppm 0.52 mg/l	585 ppm 0.78 mg/l	780 ppm 1.03 mg/l VH_2O_2
740 ppm 0.52 mg/l VH_2O	50%	60%	75%	n.A.*
1460 ppm 1.03 mg/l VH_2O	34%	43%	50%	60%
2950 ppm 2.06 mg/l VH_2O	25%	34%	43%	50%
4400 ppm 3.09 mg/l VH_2O	20%	25%	34%	43%

50 °C

$[VH_2O_2]$ / $[VH_2O]$	195 ppm 0.26 mg/l VH_2O_2	390 ppm 0.52 mg/l VH_2O_2	585 ppm 0.78 mg/l VH_2O_2	780 ppm 1.03 mg/l VH_2O_2
740 ppm 0.52 mg/l VH_2O	20%	25%	34%	43%
1460 ppm 1.03 mg/l VH_2O	11%	14,3%	20%	25%
2950 ppm 2.06 mg/l VH_2O	6%	11%	14,3%	20%
4400 ppm 3.09 mg/l VH_2O	n.A.*	6%	11%	14,3%

Fig. 2: Test matrix for the determination of D-values. Experiments were planned with four different $[VH_2O_2]$ and four different $[VH_2O]$, using H_2O_2 solutions with the indicated concentrations (in w/w) to keep the other variable constant in each case. To reduce the test effort shown (42 measurement points), D-value determinations at 30 °C and 50 °C were only performed at one concentration combination each.
* these combinations were not investigated because they either required a > 60 % H_2O_2 solution or resulted in very high D-values and therefore impracticably long process times.

tests and adjusted with a safety factor so that the flow velocity of the gas mixture is approx. 2 m/min. As the gas volume flow rate is constant, the $[VH_2O_2]$ can be adjusted via the delivered amount of H_2O_2/H_2O solution, while the ratio of $[H_2O_2]$ to $[H_2O]$ can be varied via different concentrations of the solution itself (see Fig. 2).

To measure the $[VH_2O_2]$ continously, an electrochemical gas detector of the type Dräger Polytron 7000 is used in combination with the Dräger Sensor H_2O_2 HC. The data are recorded at 1 sec intervals.

The process temperature can be set in the range from 30 °C to 60 °C via a controlled heater. The gas mixture is fed to the process chamber through a temperature-controlled heating pipe. The process chamber itself is surrounded by a temperature-controlled water jacket, which prevents condensation.

The walls of the process and preconditioning chamber are transparent to enable a visual inspection of the samples and of the chamber walls for condensation and aerosol formation.

Biological indicators

Geobacillus stearothermophilus (ATCC 7953) is used as a test germ. Several purification steps are performed on the spore suspension to remove exosporangia, residual protein and dead vegetative cells, since these would otherwise interfere with VH_2O_2-dependent inactivation by covering, or "shielding", the spores [4]. Spores are suspended in 40 % ethanol solution.

Approximately 10^6 CFU of *G. stearothermophilus* spores are inoculated onto an 8 mm diameter stainless steel disc in such a way that spores do not shield each other. The ethanol solution evaporates completely and the indicators are stored at room temperature.

All measurements are carried out with biological indicators of the same batch in order to exclude influences resulting from spore production, purification or inoculation of the spore carriers [4, 18].

D-value determination of the biological indicators

The D-values of the biological indicators are determined using the survivor curve method according to EN ISO 11138-1 [2]. The determination of the

initial population of untreated biological indicators is performed after a heat shock according to US Pharmacopoeia (15 min, 95–100 °C).

The unwrapped biological indicators are placed in the sample holder and first adjusted to 1 K above the desired process temperature in the preconditioning chamber. The loaded sample holder is then inserted into the process chamber and exposed to the sterilizing gas for the respective process time. At the end of the exposure time, the sample holder is removed from the process chamber and the samples are immediately aseptically transferred into 10 ml of chilled catalase solution (Roth, 2500 U/ml in 57 mM K_2HPO_4 buffer, pH 7). This immediately decomposes any H_2O_2 that is still adsorbed and could otherwise lead to further spore inactivation after the end of the process time.

To remove the spores from the carriers, the samples are mixed for 1 min on a test tube shaker, treated for 15 min in an ultrasonic bath (Sonorex, Bandelin) and then shaken again for 1 min. The population of the resulting spore suspension is determined by Koch's plate pouring method using TSA (tryptic soy agar, Becton, Dickinson and Company). Evaluation is performed after incubation at 57 °C for two days.

Determination of the process variables and their parameters for the D-value determination

The D-value determination by the survival curve method according to EN ISO 11138-1, Annex C, requires that exposure conditions are selected in such a way that the spore population decreases by 0.5 to 4 log levels [2]. Preliminary tests have shown that at 40 °C and a $[VH_2O_2]$ of 195–780 ppm (0.26–1.03 mg/l) these requirements are achieved within feasible process times and lead to readily countable residual populations.

In addition, the chosen settings lead to gas mixtures that are well below the calculated condensation limit at the selected temperatures [19]. Thus, avoiding local, uncontrollable condensation.

An in-depth analysis of the influences of $[VH_2O_2]$, $[VH_2O]$ and temperature on the D-value requires a vast set of experiments, as shown in the test matrix (Fig. 2). It is necessary to use H_2O_2 solutions of different concentrations. In order to somewhat reduce the high workload, measurements on the temperature dependence are initially carried out only for one $[VH_2O_2]$ (390 ppm/ 0.52 mg/l) and $[VH_2O]$ (1460 ppm/ 1.03 mg/l).

Users of different VH_2O_2 sterilization/disinfection processes sometimes use different units for the concentration of H_2O_2 and H_2O. Concentrations in VH_2O_2/plasma sterilizers are typically specified in mg/l in the sterilizer chamber. Room decontamination methods, on the other hand, often use the unit "ppm" (parts H_2O_2 per million parts in total gas phase). The VH_2O_2 sensor used here records the concentration in ppm. To allow easy readability for all potential users, concentrations are also given in mg/l. The conversion is done with the general gas equation, assuming a normal pressure of 101.3 kPa.

Results

Resistance of *G. stearothermophilus* biological indicators on stainless steel discs at constant $[VH_2O_2]$, $[VH_2O]$ and temperature in the resistometer

The germ inactivation rate, provided as a D-value, results from the semi-logarithmic plot of the surviving population versus the process time [2]. A linear progression of this survival curve is a requirement for sterilization process validations based on the half-cycle principle [1], as well as for the comparability of D-values determined by survival curve and fraction-negative (FN) methods. However, both linear and non-linear curve shapes have been reported for VH_2O_2 processes before [8, 20–22].

The method described here was used to determine survival curves, three of which are shown as examples in Fig. 3. These were measured at different $[VH_2O_2]$ and otherwise constant conditions. From the semi-logarithmic plot, a linear relationship is obtained for all test conditions. Therefore, it could be shown that the biological indicators used and the sterilization procedures described here lead to a linear survival curve. It can also be concluded that this sterilization is a first-order reaction with regard to the bacterial count under the condition that the $[VH_2O_2]$ remains constant over the time.

Dependence of the D-value on the VH_2O_2 and VH_2O concentration

In order to determine the influence of the $[VH_2O_2]$ as well as of the $[VH_2O]$

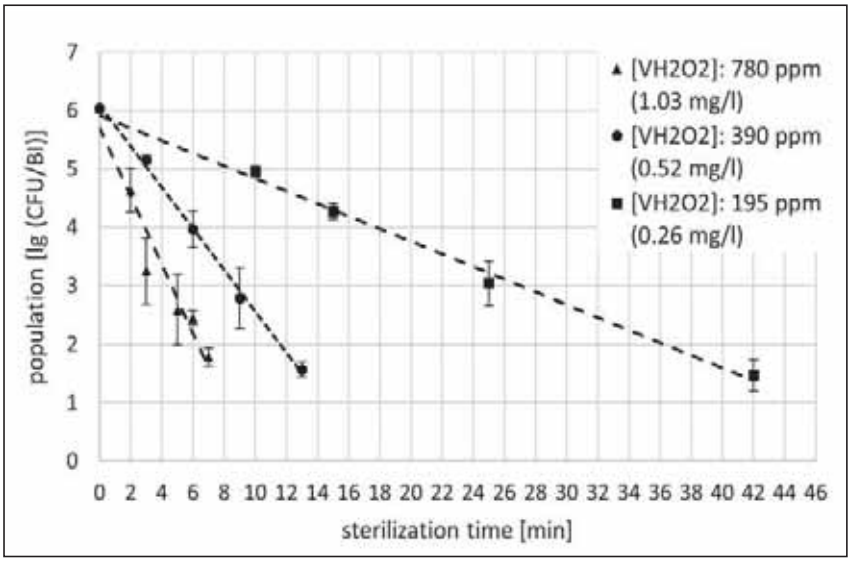

Fig. 3: Survival curves of *G. stearothermophilus* biological indicators in VH_2O_2 processes. BI of one batch were exposed to VH_2O_2 processes in the resistometer to establish survival curves according to Annex C of EN ISO 11138-1. Curves are shown that were recorded at $[VH_2O_2]$ of 195 ppm (0.26 mg/l), 390 ppm (0.52 mg/l), and 780 ppm (1.03 mg/l). The $[VH_2O]$ for all three curves was 1460 ppm (1.03 mg/l), the temperature was 40 °C, and the flow rate of the VH_2O_2-VH_2O air mixture was 2 m/min. Data points represent the mean +/- standard deviation of three independent measurements.

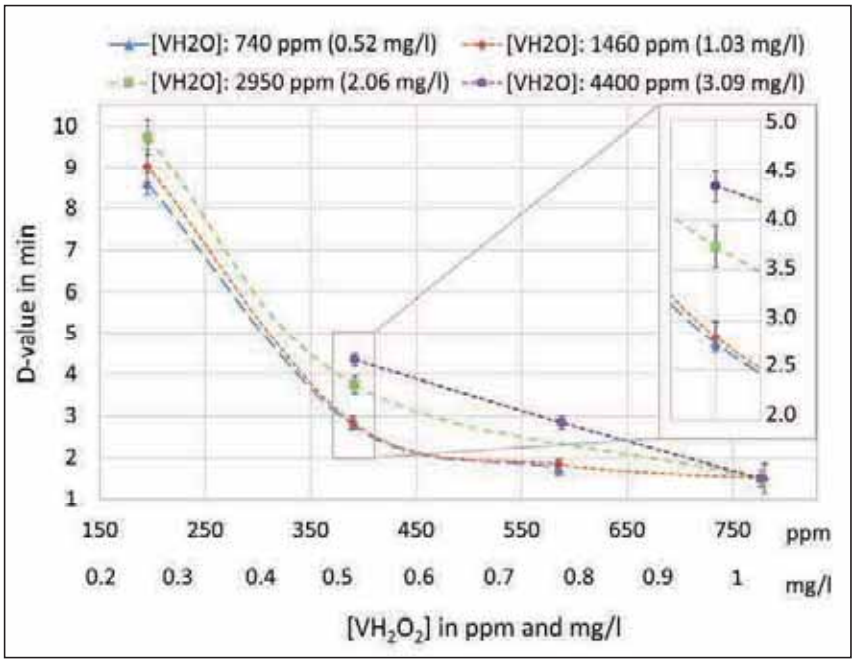

Fig. 4: Dependence of the D-value on [VH_2O_2]. Measured at 40 °C, 2 m/min gas flow and a [VH_2O] of 740 ppm (blue), 1460 ppm (orange), 2950 ppm (green) and 4400 ppm (purple). Each measurement point represents the mean +/- standard deviation of three independent D-value determinations. The inset shows the values determined for [VH_2O_2] = 390 ppm (0.52 mg/l) in more detail.

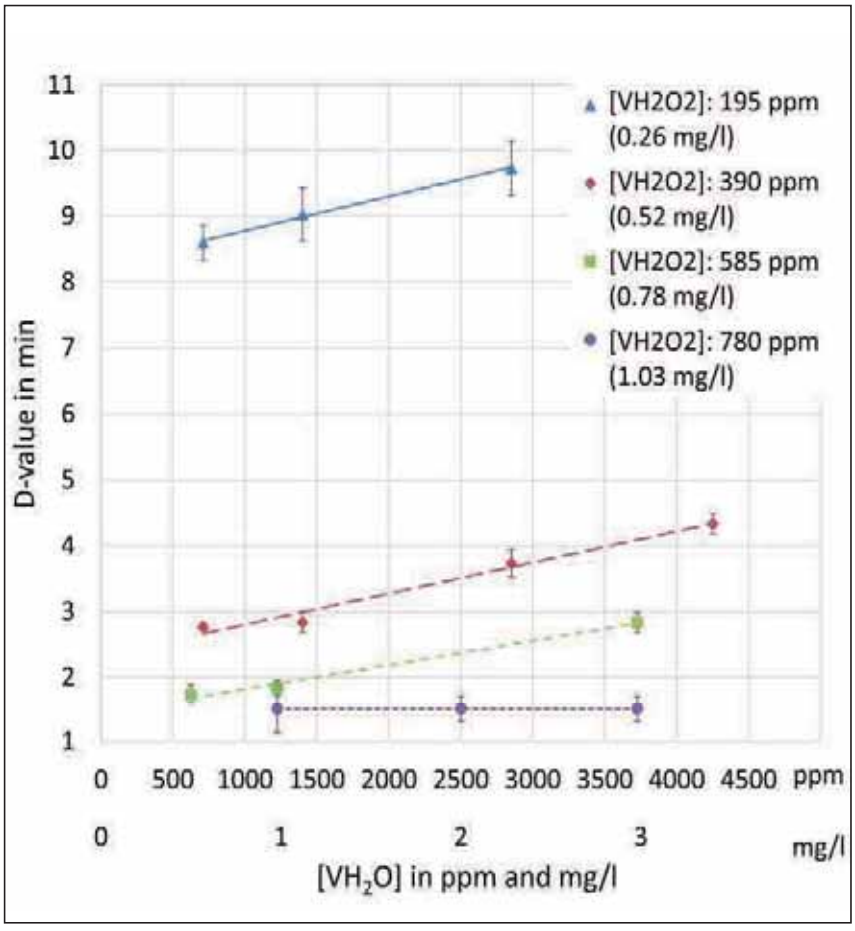

Fig. 5: Dependence of the D-value on [VH_2O]. Measured at 40 °C, 2 m/min gas flow and a [VH_2O_2] of 195 ppm (blue), 390 ppm (red), 585 ppm (green) and 780 ppm (purple). Each measurement point represents the mean +/- standard deviation of three independent D-value determinations.

on the spore inactivation rate, D-values were measured under non-condensing conditions at 40 °C and constant gas flow. The concentration combinations for H_2O_2 and H_2O listed in Tab. 1 were used.

It is obvious from Fig. 4 that the D-values of the *G. stearothermophilus* biological indicators decrease in a non-linear manner with increasing [VH_2O_2]. For example, quadrupling [VH_2O_2] from 195 ppm (0.26 mg/l) to 780 ppm (1.03 mg/l) resulted in a reduction of the D-value by approximately 83% from 9.0 min to 1.5 min at a [VH_2O] of 2950 ppm (2.06 mg/l) (Table 1, Fig. 4).

In order to analyse the influence of [VH_2O] on the D-value, measurements were performed at constant [VH_2O_2] using different concentrations of the H_2O_2/H_2O solution, resulting in different [VH_2O] in the gas phase.

Increasing the [VH_2O] leads to a measurable increase in the D-value (Tab. 1, Fig. 5). However, this effect was only observed at low [VH_2O_2]. For example, increasing the [VH_2O] sixfold while keeping the [VH_2O_2] constant at 390 ppm (0.53 mg/l) lead to an increase of the D-value from 2.8 min to 4.3 min. In contrast, at a [VH_2O_2] of 780 ppm (1.03 mg/l), the [VH_2O] had no effect on the D-value of the biological indicators used. This indicates that at [VH_2O_2] of more than 780 ppm (1.03 mg/l) the water vapor concentration plays a negligible role in germ inactivation, whereas with lower [VH_2O_2] it has a higher influence.

The results of the D-value determinations as a function of [VH_2O_2] and [VH_2O] are additionally summarized in Table 1.

Determination of the exponents for the reaction kinetics equation

The data obtained (Table 1) can now be used to determine the exponents x and y of the reaction kinetics equation:

$$\frac{-dN}{dt} = k \times N \times [VH_2O_2]^x \times -[VH_2O]^y$$

N = Number of surviving germs
k = Reaction kinetics constant (temperature-dependent)
x = [VH_2O_2] exponent
y = [VH_2O] exponent

The respective double logarithmic plot of the D-values against the concentrations can be described with a linear regression line (Fig. 6). The exponents x and y can be derived from the slope of these lines. For the exponent x for [VH_2O_2], values of 1.3 and 1.5 were determined (average: 1.4) for the water vapor concentrations listed in Tab. 2. Therefore, the spore inactivation is a reaction of approx. 1.4th order with respect to [VH_2O_2].

For the exponent y, which describes the influence of the [VH_2O], the values shown in Tab. 3 were determined. These are between 0.1 and 0.3 when the [VH_2O_2] remains low and constant. But at a [VH_2O_2] of 780 ppm (1.03 mg/l), y = 0 applies. Thus, there is no longer any influence of the [VH_2O] on the D-value detectable at higher [VH_2O_2].

Effect of the temperature on the D-value of *G. stearothermophilus* biological indicators

The dependence of the D-value on temperature is generally described as the z-value. This value describes the increase in temperature required to increase the inactivation rate tenfold. To determine the z-value for *G. stearothermophilus* biological indicators on stainless steel carriers in the VH_2O_2 process, D-values were determined at 30, 40 and 50 °C under otherwise constant conditions. Measurements were performed at [VH_2O_2] of 390 ppm (0.52 mg/l) and [VH_2O] of 1460 ppm (1.03 mg/l). From the negative reciprocal of the semi-logarithmic plot of D-values against the temperature, a z-value of 43.7 K was obtained (Fig. 7). A temperature

Tab. 1: D-values in dependence of [VH_2O_2] and [VH_2O] at 40 °C
Three independent D-value determinations were performed for each of the listed concentration combinations. The individual results of these measurements are given, as well as the mean value ± standard deviation.

[VH_2O] \ [VH_2O_2]	195 ppm 0.26 mg/l VH_2O_2	390 ppm 0.52 mg/l VH_2O_2	585 ppm 0.78 mg/l VH_2O_2	780 ppm 1.03 mg/l VH_2O_2
740 ppm 0.52 mg/l VH_2O	8.3 min 8.7 min 8.8 min	2.8 min 2.8 min 2.7 min	1.6 min 1.7 min 1.9 min	(not determined)
	Ø 8.6 ± 0.26	Ø 2.8 ± 0.06	Ø 1.7 ± 0.15	
1460 ppm 1.03 mg/l VH_2O	9.5 min 8.8 min 8.8 min	2.8 min 2.7 min 3.0 min	1.7 min 1.9 min 1.9 min	1.1 min 1.8 min 1.6 min
	Ø 9.0 ± 0.40	Ø 2.8 ± 0.15	Ø 1.8 ± 0.12	Ø 1.5 ± 0.36
2950 ppm 2.06 mg/l VH_2O	9.4 min 9.6 min 10.2 min	3.8 min 3.9 min 3.5 min	(not determined)	1.3 min 1.7 min 1.5 min
	Ø 9.7 ± 0.42	Ø 3.7 ± 0.21		Ø 1.5 ± 0.20
4400 ppm 3.09 mg/l VH_2O	(not determined)	4.3 min 4.5 min 4.2 min	2.8 min 3.0 min 2.7 min	1.3 min 1.5 min 1.7 min
		Ø 4.3 ± 0.15	Ø 2.8 ± 0.15	Ø 1.5 ± 0.20

Tab. 2: Exponent x at different [VH_2O]
Exponents are derived from the slopes of the lines in Fig. 7A.

Set [VH_2O]	Exponent x of [VH_2O_2]
195 ppm	1.5
390 ppm	1.3
585 ppm	1.3
780 ppm	1.5

Tab. 3: Exponent y at different [VH_2O_2]
Exponents are derived from the slopes of the lines in Fig. 7B.

Set [VH_2O_2]	Exponent y of [VH_2O]
195 ppm	0.1
390 ppm	0.3
585 ppm	0.3
780 ppm	0.0

increase of 43.7 K therefore leads to a reduction of the D-value by 90 %.

However, the measurements shown here were only carried out with biological indicators of one batch. From further internal experiments with other biological indicator batches, a clearly deviating z-value of approx. 26 K emerged. Therefore, further measurements must be carried out in the future with different batches and possibly also different [VH_2O_2]. This will allow more precise statements about the temperature dependency of the inactivation of different bacteria batches and different [VH_2O_2].

Discussion

In this paper, a VH_2O_2 resistometer was presented, which allows the resistance determination of "biological" indicators at constant [VH_2O_2] and [VH_2O]. The requirement of the resistometer standard EN ISO 18472 to keep all critical variables except time constant is therefore fulfilled in contrast to other previous gas phase measurements.

Using this resistometer, basic studies were performed to determine the resistance of *G. stearothermophilus* spores as a function of [VH_2O_2], [VH_2O], as well as the temperature. The results show that the inactivation rate exhibits a reaction exponent of approx. 1.4 with respect to [VH_2O_2]. This confirms the thesis published by Schaffer and Pflug in 2000, which stated that the VH_2O_2 reaction kinetics may not be of first order [22].

It is therefore obvious that for VH_2O_2 sterilization processes with non-constant [VH_2O_2] over the time, the usage of an average [VH_2O_2] value is not reasonable. For example, the calculation of the D-value according to the FN method [2, Annex D] on the basis of an average [VH_2O_2] would lead to incorrect and misleading results. Such a procedure would only be valid if the reaction kinetics were of zero-order in relation to the [VH_2O_2], i.e. if the concentration had no influence on the rate of inactivation.

The frequently used method of calculating the BI resistance based on the VH_2O_2 dose, in the form of the concentration-time integral, is only valid in the case of a first-order reaction kinetic in relation to the concentration of the sterilant. But since the VH_2O_2 sterilization is a reaction of the 1.4th order in relation to [VH_2O_2], the dose method cannot readily be used to describe the inactivation of germs and the determination of resistance, because the reaction exponent must be taken into account.

The [VH_2O_2] exponent of approx. 1.4 determined in this work is very similar to the exponent of approx. 1.5 determined by Deinhard et al. for [H_2O_2] germ inactivation in the liquid phase [5].

The reaction exponent with respect to the [VH_2O] is clearly below 1 and at higher [VH_2O_2] the spore inactivation is even independent from [VH_2O]. The negative influence of [VH_2O] on spore inactivation shown here apparently contradicts the results of Unger-Bimczok et al., who observed increased spore inactivation at higher [VH_2O] [10]. However, the measurements described there were made at much higher water vapor concentrations ranging from 3500 to 21000 ppm VH_2O and at 22 °C. Therefore, in that system, the increase in [VH_2O] led to condensation, which may be a reason for the faster spore inactivation. Interestingly, in that test series, the [VH_2O] also had no effect on the D-value at a higher [VH_2O_2] of 800 ppm. This indicates that the [VH_2O] is generally neglectable in typical VH_2O_2 sterilization processes with [VH_2O_2] ≥ 1 mg/l.

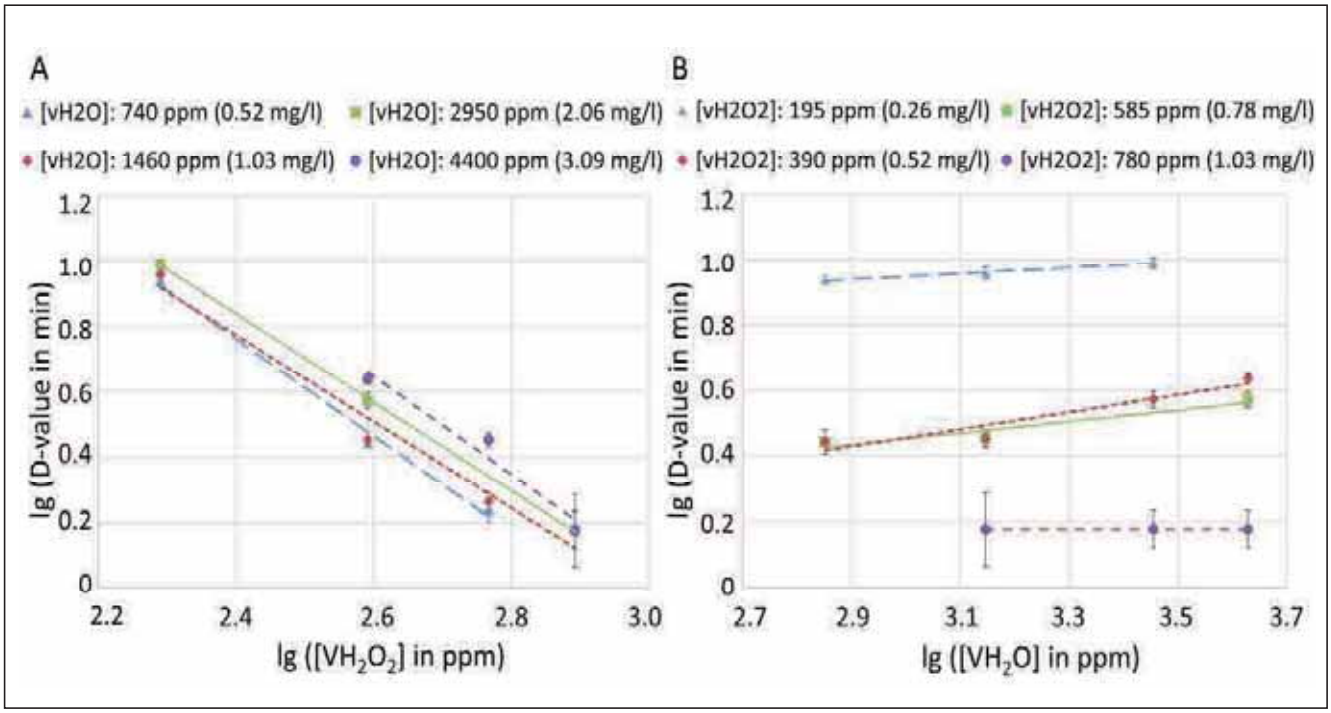

Fig. 6: Double logarithmic plot of D-values of G. stearothermophilus BI at 40 °C against [VH_2O_2] (A) and [VH_2O] (B), respectively.

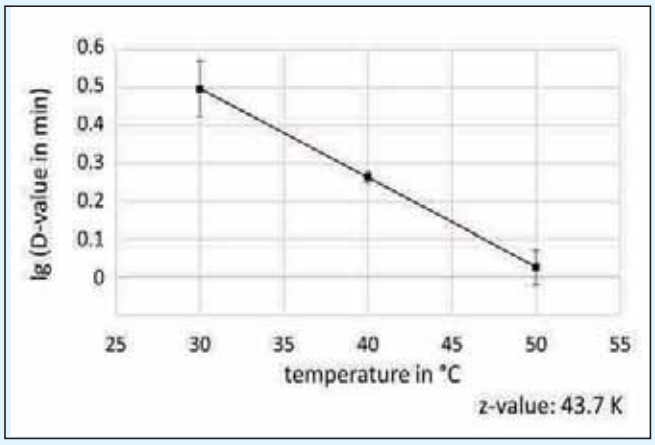

Fig. 7: Determination of the temperature-dependent z-value. Measurements were performed at 40 °C, 2 m/min gas flow with [VH_2O_2] = 390 ppm (0.52 mg/l) and [VH_2O] = 1460 ppm (1.03 mg/l). The slope of the regression line is -0.023. From this, the z-value of 43.7 K can be calculated. Each measurement point represents the mean +/- standard deviation of three independent D-value determinations.

Conclusions and perspectives

Different characteristics of biological indicators, such as cultivation conditions of the bacteria, material and nature of the spore carrier, and the type of inoculation, may have a significant influence on the D-value and z-value, especially in VH_2O_2 sterilization processes [4, 18, 23–25]. Therefore, in future experiments in the resistometer presented here, different biological indicator batches and carrier materials will be tested.

With the method presented here, it is now possible to define a reference germ required for process validation, to characterize biological indicators and also to compare them with chemical indicators, for example to determine their pass-fail window. Other applications, such as the use of self-contained biological indicators (SCBI) or the sterilization of hollow instruments, require vacuum steps and therefore cannot be performed using the method presented here. For such applications, a research sterilizer combining the VH_2O_2/VH_2O injection process with fractionated prevacuum, similar to the LTSF sterilization process [26], would be suitable. This could circumvent low penetration in hollow devices, which is a problem with many of the current VH_2O_2 processes using one deep vacuum per injection.

The results of this work provide basic information on the inactivation of germs in VH_2O_2 sterilization processes and can thus contribute to improving the evaluation of these processes.

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