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Multiple sensor-type system for monitoring the microbicidal effectiveness of aseptic sterilisation processes

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ABSTRACT

The present work describes a novel multiple sensor-type system for the real-time analysis of aseptic sterilisation processes employing gaseous hydrogen peroxide (H_2O_2) as a sterilant. The inactivation kinetics of *Bacillus atrophaeus* by gaseous H_2O_2 have been investigated by means of a methodical calibration experiment, taking into account the process variables H_2O_2 concentration, humidity and gas temperature. It has been found that the microbicidal effectiveness at H_2O_2 concentrations above 2% v/v is largely determined by the concentration itself, while at lower H_2O_2 concentrations, the gas temperature and humidity play a leading role. Furthermore, the responses of different types of gas sensors towards the influencing factors of the sterilisation process have been analysed within the same experiment. Based on a correlation established between the inactivation kinetics and the sensor responses, a calorimetric H_2O_2 sensor and a metal-oxide semiconductor (MOX) sensor have been identified as possible candidates for monitoring the microbicidal effectiveness of aseptic sterilisation processes employing gaseous H_2O_2. Therefore, two linear models that describe the relationship between sensor response and microbicidal effectiveness have been proposed.

1. Introduction

The origins of aseptic processing can be dated back to more than a century. Nowadays, it represents a key-technology in modern food processing and was recently ranked the No. 1 innovation in food technology (Floros et al., 2010). The core elements of aseptic processing are the thermal treatment of the product to be filled, the sterilisation of the container and the filling and sealing in a recontamination free environment in such way that it maintains sterility (von Bockelmann & von Bockelmann, 1986). In the course of this, the sterilisation of the packaging material is an important criterion. Modern filling machines use hydrogen peroxide (H_2O_2) as a sterilant for carton packages.

Previously applied in liquid phase, gaseous H_2O_2 today established as the sterilising agent of choice, since it demonstrated a higher activity compared to aqueous H_2O_2 solution (Cerny, 1992; McDonnel & Russell, 1999; McDonnell, 2007; Wang & Toledo, 1986). It is believed that the microbicidal activity of H_2O_2 is based on the activity of products derived from its decomposition. It has been demonstrated that H_2O_2 serves as a source of highly reactive species, above all hydroxyl radicals, which cause damage to a variety of microorganisms including highly resistant spores (Heckert et al., 1997; Klapes & Vesley, 1990; Kokubo, Inoue, & Akers, 1998; Toniolo, Geatti, Bontempelli, & Schiavon, 2001). The latter are used as test organisms for determining the sterilisation effectiveness in the context of the so-called challenge test: *Bacillus atrophaeus* is the recommended test organism for sterilisation processes employing H_2O_2 in combination with heat, since it inherently has a high resistance against the sterilisation medium (VDMA, 2008). An approved test method is the count-reduction test (CRT). The CRT is carried out using test packages inoculated with an unnaturally high load (at least 10^5) of *B. atrophaeus*, which are taken to the sterilisation process. The decrease in viable spore count after the sterilisation serves as a measure for the effectiveness of sterilisation. It is usually expressed by orders of magnitude, the so-called logarithmic cycle reduction (LCR). Although the CRT represents a recognised method, its major disadvantage is the elaborately preparation and post-processing of samples. Solely, the incubation period of the spores in ideal case amounts to at least

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24 h. Thus, microbiological tests, like the CRT, cannot be used by means of a real-time monitoring.

It is the aim of this study, to characterise different influencing factors of the sterilisation with gaseous H_2O_2 by means of the CRT with *B. atrophaeus*. As such, the H_2O_2 concentration, humidity and temperature have been identified by previous studies (Forney, Rij, Denis-Arrue, & Smilanick, 1991; Kirchner et al., 2013; Kokubo et al., 1998; Reisert, Geissler, Flörke, Näther, et al., 2011; Reisert et al., 2012; Unger-Bimczok, Kottke, Hertel, & Rauschnabel, 2008). Moreover, the response of multiple types of gas sensors, namely a calorimetric H_2O_2 sensor and different commercially available metal-oxide semiconductor (MOX) gas sensors towards the influencing factors of the sterilisation by gaseous H_2O_2 have been investigated. For this purpose, a methodical calibration experiment, allowing a detailed investigation of the impact of each of the mentioned influencing factors, has been elaborated. This way, the inactivation kinetics of *B. atrophaeus* and the sensor responses have been analysed for a broad parameter range of the variables H_2O_2 concentration (1.67–4.32% v/v), H_2O concentration (15.10–22.60% v/v) and gas temperature (150–330 °C).

Based on the results of the calibration experiment, a correlation between the microbiological effectiveness and the response of the gas sensors has been established. In the end, two different models will be proposed. One is related to the calorimetric gas sensor and covers predominantly the range of higher H_2O_2 concentrations (>2% v/v), while the second one is related to a metal-oxide semiconductor gas sensor and may be applied at rather low H_2O_2 concentrations (<2% v/v). It will be shown that, by the right choice of sensors in combination with appropriate mathematical models, a sensor system for the real-time monitoring of sterilisation processes employing gaseous H_2O_2 can be realised.

2. Materials and methods

2.1. Experimental set-up

The test apparatus that has been used to investigate the inactivation kinetics of *B. atrophaeus* and to characterise the gas sensors conforms to a sterilisation unit of an industrial aseptic filling machine for large-scale production of food packages and has been described in detail in previous publications (Kirchner et al., 2010; Näther et al., 2006). In short, the test apparatus consists of a heater, two dosing pumps for hydrogen peroxide solution and water, a flow control unit that provides a steady flow of compressed air and a measuring chamber to which the sterilising gas is fed by four gas nozzles. To adjust a certain concentration of H_2O_2 in the measuring chamber, the provided air stream is fed with an aqueous hydrogen peroxide solution of 35% w/w. In order to simulate the utilisation of lower concentrated H_2O_2 solutions, the air stream can additionally be fed with water. The mixture of air and aqueous H_2O_2 solution will subsequently be evaporated in the heater. The evaporated mixture is further heated up to the adjusted temperature and then equally dispensed to the measuring chamber by four gas nozzles. Inside the chamber, the measuring point (MP) for the sensors and microbiological tests was chosen at a distance of 5.2 cm each under a separate nozzle. While the sensors were positioned firmly under one nozzle, the microbiological samples were introduced via a time-controlled hydraulic slide. To ensure that the measurements are transferable to relevant applications, the measuring point was chosen similarly to the test point of industrial processes. Also, in order to obtain information on the true gas temperature at the MP, an additional temperature sensor has been installed at this point. A schematic of the experimental set-up is depicted in Fig. 1.

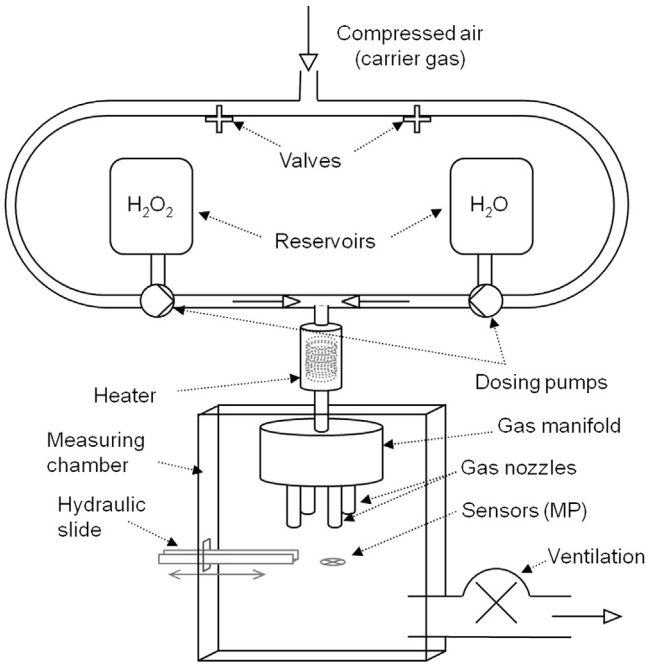

Fig. 1. Schematic of the experimental set-up (modified from Reisert, Geissler, Flörke, Wagner, et al., 2011).

2.2. Microbiological tests

The microbiological tests have been carried out according to the count-reduction test (CRT) (VDMA, 2008). As test samples, *B. atrophaeus* spores inoculated on aluminum strips were used. The initial bacterial count was on average at $2.04*10^6$ cfu (colony forming units) per strip. After exposure to the sterilising gas, the test strips have been stored in 10 ml Ringer's solution. Afterwards, the spores were stripped off the aluminium strip in an ultrasonic bath and plated on nutrient agar. To ensure that a countable number of bacterial colonies (<300) are grown on the agar plates, several dilutions of the spore suspension have been prepared prior to the plating. The inoculation time of the plates was 24 h at 37 °C. For each setting of the machine parameters, ten microbiological samples have been taken. According to the CRT, the LCR can be calculated as follows:

$$\mathrm{LCR} = \log(N_0) - \log(N_S) \quad (1)$$

Here, N_0 is the initial count of *B. atrophaeus* prior to the sterilisation, which was equal to $2.04*10^6$ cfu and N_S is the averaged final count of ten samples after the sterilisation process.

2.3. Gas sensors

A calorimetric-type gas sensor for the detection of hydrogen peroxide, which has been developed in the authors' laboratory and four commercially available MOX sensors have been investigated.

The calorimetric H_2O_2 sensor was previously described in detail by several publications of the authors (Kirchner, Reisert, & Schöning, 2014; Reisert et al., 2010). In short, it consists of a differential set-up of two temperature sensors, wherein one of the temperature sensors is catalytically activated by MnO_2 powder and the second one is passivated by a perfluoroalkoxy (PFA) coating. In presence of hydrogen peroxide, a temperature difference between the catalytically activated and the passivated sensor, caused by the exothermal reaction of hydrogen peroxide on the catalyst, can be