Food Control 31 (2013) 530–538

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Monitoring the microbicidal effectiveness of gaseous hydrogen peroxide in sterilisation processes by means of a calorimetric gas sensor

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ARTICLE INFO

Article history:
Received 23 July 2012
Received in revised form
20 November 2012
Accepted 27 November 2012

Keywords:
Hydrogen peroxide
Sterilisation
Bacillus atrophaeus
Calorimetric gas sensor

ABSTRACT

In the present work, a novel method for monitoring sterilisation processes with gaseous H_2O_2 in combination with heat activation by means of a specially designed calorimetric gas sensor was evaluated. Therefore, the sterilisation process was extensively studied by using test specimens inoculated with *Bacillus atrophaeus* spores in order to identify the most influencing process factors on its microbicidal effectiveness. Besides the contact time of the test specimens with gaseous H_2O_2 varied between 0.2 and 0.5 s, the present H_2O_2 concentration in a range from 0 to 8% v/v (volume percent) had a strong influence on the microbicidal effectiveness, whereas the change of the vaporiser temperature, gas flow and humidity were almost negligible. Furthermore, a calorimetric H_2O_2 gas sensor was characterised in the sterilisation process with gaseous H_2O_2 in a wide range of parameter settings, wherein the measurement signal has shown a linear response against the H_2O_2 concentration with a sensitivity of 4.75 °C/(% v/v). In a final step, a correlation model by matching the measurement signal of the gas sensor with the microbial inactivation kinetics was established that demonstrates its suitability as an efficient method for validating the microbicidal effectiveness of sterilisation processes with gaseous H_2O_2.

1. Introduction

The sterilisation of packaging material is an essential part of an aseptic food filling system in order to achieve an extended shelf life of packed products (especially low-acid food such as milk) on the one hand, and to obviate the transmission of pathogenic microorganisms to the consumers on the other hand (Gould, 1996). In general, the process of sterilisation should possess a rapid and reliable microbicidal effectiveness so that the potential number of viable microorganisms on the package surface is entirely inactivated (Ansari & Datta, 2003; Moruzzi, Garthright, & Floros, 2000). Furthermore, it should be compatible with the packaging material, easily removable from the package surface, and the unavoidable residue of the sterilisation agent should not affect the product and should be harmless for the consumer (Ansari & Datta, 2003).

In aseptic food technology, hydrogen peroxide (H_2O_2) is the most commonly used sterilisation agent for food packaging material at present time. Amongst its benefits, H_2O_2 at low concentration levels of residue is not toxic and it has a highly microbicidal effectiveness against a broad spectrum of microorganisms, such as bacteria, spores, viruses, fungi and yeast (Block, 1991; Heckert et al., 1997), that is enhanced by a physical process of either heat or UV radiation (Bayliss & Waites, 1982; Smith & Brown, 1980; Toledo, Escher, & Ayres, 1973). The mechanism of the microbial inactivation seems to rely on the fact that H_2O_2 serves as an oxidant by generating free hydroxyl radicals as intermediates during its physically induced decomposition (McDonnell & Russell, 1999). These radicals damage cell components, like proteins, lipids and DNA (Block, 1991; Russell, 1990). In general, the microbicidal effectiveness is significantly increased by gaseous H_2O_2 (McDonnell & Russell, 1999).

For the validation and control of the sterilisation's effectiveness, microbiological challenge tests have to be carried out. In

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http://dx.doi.org/10.1016/j.foodcont.2012.11.048

these tests, artificially inoculated package material with bacteria spores, which are highly resistant against the sterilisation agent, are exposed to the sterilisation process, afterwards, incubated for a defined time and finally, either the number of survived spores in form of grown bacterial colonies are counted (count-reduction test) or the relation between sterile and unsterile packages is determined (end-point test) (Cerny, 1992). Even though, these procedures represent well-established and reliable validation methods of the sterilisation process that is indispensable for its inspections in defined intervals, they have some disadvantages: the methods are time- and labour-consuming; continuous controlling is not possible and the results are subject to statistical fluctuations. Due to these facts, there is a great demand of the aseptic food industry for a method that additionally allows the determination of the microbicidal effectiveness of the sterilisation process continuously in-line with low operational costs. Supposed that the sterilisation's effectiveness predominantly depends on the amount of gaseous H_2O_2, a sensor system is required for quantitatively measuring the gaseous H_2O_2 concentration under harsh environmental conditions of the sterilisation process and thus, this system could help to verify the sterilisation's effectiveness in-line.

The objective of this study can be divided into three steps. In a first step, the microbicidal effectiveness of gaseous hydrogen peroxide at elevated gas temperatures is evaluated for various settings of contact time, gas velocity, humidity and especially, of the gaseous H_2O_2 concentration, whereby the predominant effect of the gas concentration on the effectiveness will be demonstrated. Therein, the chosen parameter ranges correspond to real, industrial sterilisation processes. In a second step, the actual H_2O_2 concentration is quantitatively measured by a novel gas sensor at same settings of the process parameters as for the determination of the sterilisation's effectiveness. The H_2O_2 gas sensor module is based on a calorimetric differential set-up that was first introduced by Näther, Henkel, Schneider, and Schöning (2009). In a final step, a correlation model between the microbicidal effectiveness and the measurement signal of the calorimetric gas sensor is established.

As a result, the present study should demonstrate that the use of such a calorimetric H_2O_2 gas sensor and the development of a correlation model between the sensor's signal and the microbicidal effectiveness are representing a novel method to continuously monitor industrial sterilisation processes.

2. Materials and methods

2.1. Sterilisation test rig

In a developed test rig, already introduced in Kirchner et al. (2010) and Näther et al. (2006), the sterilisation process with gaseous hydrogen peroxide at elevated gas temperature was reproduced. The test rig contains a dosing system with two piston pumps, where one of them serves for the H_2O_2 solution (35% w/w, from FMC Industrial Chemicals) and the other one for deionised water. A gas flow of compressed air controlled by a flow meter and a regulation valve was used as carrier gas for both liquids. Furthermore, the test rig includes a vaporisation unit built up by two heating elements in series. The heating power of the heating elements is controlled by the measured temperature of the gas stream at the outlet nozzle of the vaporisation unit. The microbiological specimens and the calorimetric gas sensor have been placed via a hydraulic slide rail in a defined distance – similar as for industrial processes with carton packages – underneath the gas-outlet nozzle in an aseptic chamber of the test rig.

2.2. Calorimetric gas sensor

For measuring the gaseous H_2O_2 concentration, a calorimetric gas sensor has been implemented in the aseptic chamber of the sterilisation test rig. The sensor principle is based on a calorimetric differential setup, which consists of two temperature-sensitive thin-film resistors, wherein one of them is covered by a polymeric passivation layer (here, SU-8 photoresist) and the second one is additionally coated by a catalytically active dispersion of manganese(IV) oxide (s. Fig. 1). If the calorimetric gas sensor is exposed to a H_2O_2 gas stream, a temperature difference between the catalytically activated (active sensor segment) and the passivated thin-film resistor (passive sensor segment) caused by an exothermal decomposition of hydrogen peroxide on the catalytic surface correlates with the present H_2O_2 concentration in the gas-phase and yielding a measurement signal according to Eq. (1):

$$\Delta T_{\text{signal}} = S \cdot c_{H_2O_2} + \Delta T_0 \quad (1)$$

Herein, S is the sensor's sensitivity, $c_{H_2O_2}$ is the H_2O_2 concentration and ΔT_0 is the sensor's off-set.

Before the sensor was exposed to the H_2O_2 gas stream, the resistors of the sensor were calibrated in a temperature range between 10 °C and 85 °C in steps of 5 °C in a thermostat (RE 207 from LAUDA) in order to precisely detect the temperature on the active and passive sensor segment, respectively. The fabrication procedure of the thin-film sensor and its response mechanism were already presented in Kirchner et al. (2011) and Kirchner et al. (2012) in detail.

2.3. Microbiological test specimens

A spore suspension of *Bacillus atrophaeus* ATCC 9372 was used for the microbiological tests of the sterilisation process. Spores of *B. atrophaeus* are recommended for testing H_2O_2 sterilisation processes due to their high resistance against H_2O_2 (VDMA, 2008). The spore suspension has an initial load of $8 \cdot 10^8$ cfu/ml (cfu: colony forming units) in ethanol solution (70%). The test specimens, each of them made up of a flat aluminium plate with a size of

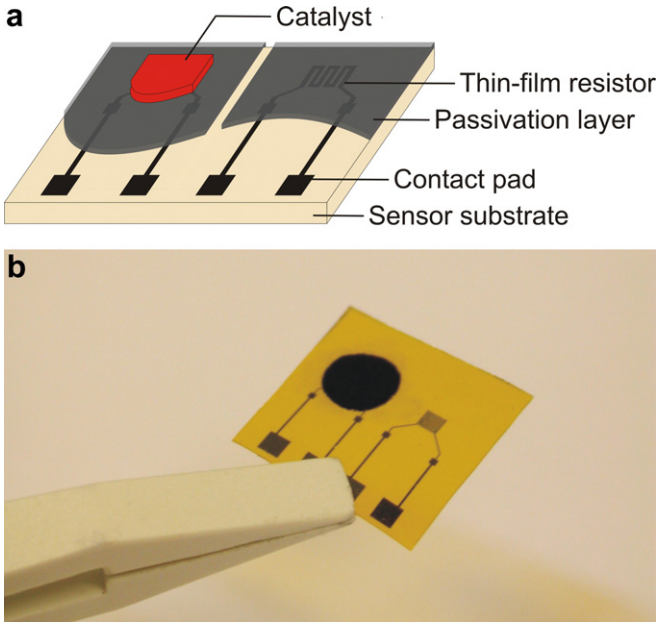

Fig. 1. a) Scheme of the sensor set-up with two thin-film resistors, passivation layer and catalyst and b) calorimetric H_2O_2 gas sensor on a polyimide substrate (sensor size: 10×10 mm^2).