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EHEDG Update

A method for assessing the bacterial retention ability of hydrophobic membrane filters

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This paper is the 19th in a series of articles on the hygienic design of food processing equipment. It is based on a report prepared by the Test Methods Subgroup of the European Hygienic Equipment Design Group (EHEDG). A method for validating the bacterial retention ability of sterilizing grade hydrophobic membrane filters is described. The procedure was developed at The TNO Nutrition and Food Research Institute, Zeist, The Netherlands. The bacterial aerosol challenge test was found to be sufficiently sensitive to determine filter efficiency up to 99.9995% for the types of micro-organisms used.* © 2000 Elsevier Science Ltd. All rights reserved.

Introduction

Recent research has found that hydrophobic membrane filters with a pore size of 0.22 μm, do not retain micro-organisms under all process conditions. This paper describes a method of evaluating the performance of such filters under a range of operating conditions. The main research was carried out at TNO Nutrition and Food Research Institute, Zeist, Netherlands, in close cooperation with filter manufacturers.

A bacterial aerosol challenge filter test (TBAC) was developed. The filter to be tested was then challenged with an aerosol of bacteria for some time. During that period bacteria must not pass through the filter. The method was used to qualify filter systems for air filtration and exhaust gas filtration on fermenters. In these applications filters are intended to prevent micro-organisms from contaminating the environment.

Materials

The aerobic bacterium *Brevundimonas diminuta* and the aerobic bacterium *Bacillus subtilis* were selected as test organisms.

Brevundimonas diminuta ATCC 19146 has a diameter of 0.5 to 0.8 mm and is therefore used to test filter retention ability. This strain is widely used as a test organism in liquid filtration. Spores of *Bacillus subtilis* ATCC 9372 have a diameter of about 1 μm and are used to test filter retention ability as they are less affected by drying during aerosolization.

Bs. diminuta is grown in broth in a rotary shaker incubator at 30°C for 48 h. After incubation, 300 ml of this culture is centrifugated and the pellet is suspended into 400 ml sterile demineralised water, prior to each experiment. The total amount of colony forming units (cfu) is determined at the beginning and the end of each bacterial challenge test on Nutrient Agar plates which are incubated at 30°C for 48 h. Filter permeating bacteria are detected in the exhaust-gas of the filter on Nutrient Agar plates using a three stage Microbial Impactor Sampler. The plates in the sampler are changed every 15 min in a biohazard laminair flow cabinet and are incubated at 30°C for 48 h.

Bacillus subtilis is grown in a rotary shaker incubator at 30°C for 48 h. The culture is heated at 80°C for 10 min to kill the vegative cells. 300 ml of the culture is centrifugated, the pellet washed and re-suspended in demineralized filtered (0.22 μm) water three times to remove broth and cell debris from the spores. The pellet is suspended into 400 ml sterile demineralized water prior to each experiment. The total amount of colony forming units (cfu) is determined at the beginning and the end of each test on Nutrient Agar plates, which are incubated at 30°C for 48 h.

* Readers requiring further information on the EHEDG are referred to *Trends in Food Science & Technology* (1992) 3(11), 277.

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Detection

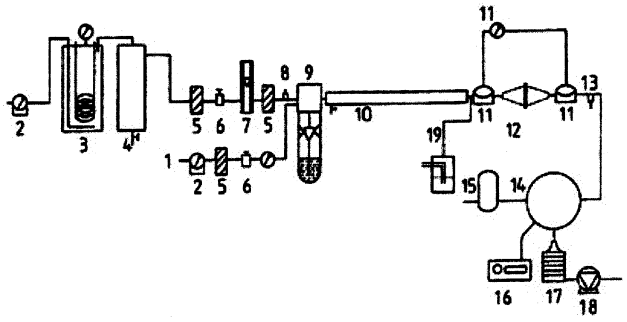
Filter permeating bacteria are detected downstream of the filter on Nutrient Agar plates (Oxoid) using a three stage Microbial Impactor Sampler. The plates in the sampler are changed every 15 min in a biohazard laminair flow cabinet and are incubated at 30°C for 48 h.

Equipment

- Collison nebulizer, specific particle size range 0.5–2 μm (>90%); Domnick Hunter UK
- Microbial Impactor Sampler (MIS); Landre Intechmij B.V., The Netherlands.

Test procedure

Each filter to be tested is taken directly from the original package and placed in the test system. A schematic diagram of a test system used for testing bacterial impermeability is shown in Fig. 1. Depending on the filter size, the filter to be tested is challenged with an air flow of 50–400 l/minute at a relative humidity of 40–99% (not condensing) containing a bacterial challenge of $>10^7$ cells/cm^2 filter area, a total of 10^{11} cells per test.



1 inlet compressed air 6 bar
2 reducing valve
3 water evaporator with heating spiral and connected water bath 20–25°C
4 condensor
5 sterilizing grade filter
6 stopcock
7 calibrated flowmeter
8 steam connection
9 Collison nebulizer, working pressure 2 bar
10 evaporating section 40°C
11 pressure difference meter
12 holder for filter to be tested
13 sensor for temperature and relative. humidity
14 sampling cabinet
15 sterilizing grade filter for gas outlet
16 laser particle counter, six channels
17 microbial impactor sampler, three stages
18 vacuum pump ±30 l/min
19 foam generator

Fig. 1. Bacterial retention test system.

The test procedure consists of two steps:

- a zero control test;
- a bacterial challenge test.

Zero control test

Prior to each bacterial challenge test, a filter is controlled for damages (leakages, pin holes) by a Wet Integrity Test. This test is performed by applying air pressure to a prewetted filter (isopropanol:water mixture, 60:40). When the wetted filter is pressurized the pressure decay, diffusional flow and the bubble point are measured (Sartocheck II or Palltronic FFE04). The results of these measurements must be within specifications for the filter. After drying overnight at 55°C the filter is placed in the test system. The whole system is then treated with saturated steam at 121°C for 30 min. The system is dried and cooled by using dry prefiltered (0.22 μm hydrophobic air-filter) sterile air. The test is undertaken by aerosolizing sterile water for 30 min in the upstream airflow with microbial sampling in the upstream and downstream airflow every 15 min using the MIS.

The zero control test is carried out on three filters of the same type from three different lot numbers.

Bacterial challenge test procedure

After controlling the filter by a Wet Integrity Test and drying the filter overnight at 55°C, the filter is placed in the test rig. The complete system is treated with saturated steam at 121°C for 30 min.

The system is dried and cooled by using dry sterile air. The test starts with a zero test by nebulizing sterile water for 30 min in the upstream airflow, microbial sampling downstream every 15 min using the MIS. The bacterial challenge test is undertaken by nebulizing the test microorganism for 30 min in the upstream airflow with microbial sampling in the upstream and downstream airflow every 15 min using the MIS. At the end of the bacterial challenge test the filter is removed from the test rig and controlled by a Wet Integrity Test.

The bacterial challenge test is carried out on three different filters of the same type.

Interpretation of results

The filter must pass all tests with no bacteria being detected downstream of the filter. The total bacterial loading on the filter is calculated by multiplying the lowest count of the bacterial suspension with its nebulized volume and should be $\geq 10^7$ cells per cm^2 filter area. The MIS is only sampling a portion from the total airflow downstream of the filter. Therefore the measured data have to be multiplied by a factor calculated from the total airflow divided by the sample volume of the MIS.

Discussion

The bacterial aerosol bacterial challenge test is sufficiently sensitive to determine filter efficiency up to 99.9995% for the used types of microorganisms.

The test is carried out on three different filters of the same type. The filter must pass all control and microbial filter integrity tests.

Acknowledgements

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This paper presents guidelines recommended by the Test Methods Subgroup of the European Hygienic Equipment Design Group (EHEDG). Copies of the full report (EHEDG Doc. 19) by K. Anderson, T. Bénézech (Chairman), B. Carpentier, G.J. Curiel, K. Haugan, J. Kastelein, R.D. Mackintosh, E. Marquis, U. Ronner, A.W. Timperley and G. Wirtanen are available from pubs@campden.co.uk. For information about the EHEDG, visit the website: www.ehedg.org.

Further reading

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