Technical / Analytical Committee

of the European Association of Depth Filtration

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Test methods for Depth Filter Media

Filter Sheets

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Contents

1. General data

1.1. Substance (basis weight)

Purpose: Examination of a product paramater

Principle: Weighing of a depth filter sheet with defined area

Equipment: Balance (Sensitivity 0.01 g)
Punching tool
Drying cabinet, 106 ± 1 °C
Desiccator with silica gel

Procedure:

- Die-cutting of approx. 100 cm^2 (with an accuracy of 0.1 cm^2) from the test sample
- Drying at 106° C up to constant weight
- Cooling down in the desiccator to room temperature
- Weighing

Calculation:

$$\text{Substance}\,[g\,m^{-2}] = \frac{\text{Mass}\,[g]}{\text{Area}\,[m^2]}$$

Results: Results to be indicated in gm^{-2} rounded off to 1 g m^{-2}

Literature: in accordance with DIN 54 540 part 2

1.2. Thickness

Purpose: Examination of a product parameter

Principle: The thickness for depth filter sheets is the distance between top and bottom side of a depth filter sheet sample, measured as vertical distance of two plane parallel measuring surfaces between which the sample is under a certain contact pressure

Equipment: Commercial caliper gauge (for paper and cardboards)

Procedure:

- Bearing surface 10 - 50 cm^2
- Lying plane
- Mean value of three individual measurements
- Bearing pressure 2 kNm^{-2} = 2000 Pa (200 g cm^{-2})

Results: The results are to be rounded off to 0.1 mm

Literature: with reference to DIN 54 540 part 3

1.3. Bulk density (weight of unit volume)

Purpose: Examination of a product parameter

Principle: Obtaining the quotient of 1.1. Substance (basis weight) and 1.2. Thickness

Calculation:

$$\text{Bulk density } [\text{g m}^{-3}] = \frac{\text{FG [g]}}{10{,}000\ \text{cm}^2 \cdot \text{h [cm]}}$$

FG (g): Mass of the dried test sample per m²
h (cm): Thickness of the test sample

Results: Results to be indicated in g cm^{-3} rounded off to 0.01 g cm^{-3}

Literature: with reference to DIN 53 105 part 1

1.4. Ash content

Purpose: Determination of the matters volatile at 900° C (e.g. organic substances)

Principle: The ash content is the residue remaining after burning of the sample at a fixed temperature. The ash content is indicated as mass portion in % related to the oven-dried sample.

Equipment: Oven, 900 ± 25 °C
Analytical balance (sensitivity 0.1 mg)
Porcelain resp. platinum crucible
Desiccator, with silica gel

Procedure:

- About 3 g of dried depth filter sheet are weighed - exact to 0.1 mg - in a constantly glowed and weighed crucible
- Glow at 900° C up to constant weight
- Cool down to room temperature in the desiccator and weigh back

Calculation:

$$\text{Ash content [\%]} = \frac{(A - B) \cdot 100}{(B - C)}$$

A: Crucible, full, after glowing
B: Crucible, full, prior to glowing
C: Crucible, empty

Results: The results are to be rounded off to 0.1 %

Literature: with reference to DIN 54 370

2. Purity test

Purpose: The content of extractable cations of depth filter sheets has an influence on the quality of the product to be filtered.

Principle: Extraction of the depth filter sheet under defined conditions
Determination of selected cations in the extract.

2.1. Extraction of the depth filter sheets

Equipment: Laboratory filter (effective filter area > 128 cm^2)
Hose pump or gear pump

Reagents: 5 % acetic acid p.a.

Procedure:

- Install the fillter holder horizontally. For a better venting the direction of filtration is from bottom to top
- Volume flow V = (500 ± 50) L m^{-2} h^{-1}
- Starting volume 25 Lm^{-2}
- The starting volume is constantly filtered in circuit until 100 Lm^{-2} have been pumped through the depth filter sheet (with V = 500 L m^{-2} h^{-1} the filtration time is exactly 12 minutes)
- In case of drip losses these are collected and added to the total volume after filtration
- At the end of the filtration time the elution is finished. Emptying of the filter with pressure is not carried out
- Determination of the corresponding cations in the filtrate

Application examples:

- d = 14 cm: A_{eff} = 128 cm²
  V = 320 mL
  V = 106 ± 10.6 mL min^{-1}

- d = 6 cm: Aeff = 20 cm²
  V = 50 mL
  V = 16.7 ± 1.7 mL min^{-1}

Literature: With reference to FDA and Recommendation XXXVI/1 of the Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin - BgVV - (Federal Institute for Health Protection of Consumers and Veterinary Medicine)

2.2. Determination of iron

2.2.1. Photometric measuring method with 1.10-phenanthroline monohydrate

Equipment: Photometer with λ = 505 nm
10 mm bulbs
Water bath, 60° C
50 mL Erlenmeyer flask of PE
25 mL pipette
2 mL pipette

Reagents: 1.10-phenanthroline monohydrate, c = 3 gL-1
ascorbic acid p.a.

Procedure:

- Pipette 25 mL of filtrate into a 50 mL Erlenmeyer flask
- Add 2 mL of 1.10-phenanthroline-monohydrate solution
- Add one point of a spatula of ascorbic acid (about 25 mg)
- Preparation of the blank value (instead of phenantroline solution 2 mL of demin H_20)
- Shake
- Then heat the sample in a water bath for 15 min to 60° C
- After cooling down determination of the extinctions in 10 mm bulbs at λ = 505 nm againgst demin. H_2O
- If the extinctions are not within the Lambert-Beer Law, dilutions are to be made

Calculation:

Determination of the measured values in mgL-1 from a pre-established calibration curve

$$c\,[\mathrm{mg\,kg^{-1}}] = \frac{c\,[\mathrm{mg\,L^{-1}}] \cdot 10{,}000\ \mathrm{cm^2} \cdot V\,[\mathrm{L}]}{A_{eff}\,[\mathrm{cm^2}] \cdot FG\,[\mathrm{kg}]}$$

c (mg kg^{-1}): Concentration of the extracted cation in the filter sheet
c (mg L^{-1}): Measured value at the cation determination in the filtrate

Aeff (cm^2): Effective filter area during determination
FG (kg): Weight of the dried test sample per m^2
V (L): Actual rinsing volume during determination

Results: Results to be indicated in mg kg^{-1} rounded off to 1 mg kg^{-1}

2.2.2. AAS flame technique

Equipment: AAS flame technique
100 mL volumetric flask for standard solutions
Pipettes

Reagents: Fe stock solution 1000 mgL-1
5 % acetic acid p.a.

Equipment parameters:
Wave length = 248.3 nm
Burner gas = air/acetylene

Procedure:

- Adjustment of the Fe-specific data at the AAS in accordance with the instructions for use of the measuring unit.
- Preparation of at least two standard solutions of different concentration within the measuring range from the stock solution using the 5 % acetic acid as solvent
- Calibration of the AAS in mg L^{-1}
- Determination of the Fe concentration in mg L^{-1} in the filtrate

Calculation:

$$c\,[\mathrm{mg\,kg^{-1}}] = \frac{c\,[\mathrm{mg\,L^{-1}}] \cdot 10{,}000\ \mathrm{cm^2} \cdot V\,[\mathrm{L}]}{A_{\mathrm{eff}}\,[\mathrm{cm^2}] \cdot FG\,[\mathrm{kg}]}$$

c (mg kg^{-1}): Concentration of the extracted cation in the filter sheet
c (mg L^{-1}): Measured value at the cation determination in the filtrate
Aeff (cm²): Effective filter area during determination
FG (kg): Weight of the dried test sample per m²
V (L): Actual rinsing volume during determination

Results: Results to be indicated in mg kg-1 rounded off to 1 mg kg-1

2.3. Determination of calcium

2.3.1. Volumetric measuring method with Titriplex III

Equipment: Burette
250 ml Erlenmeyer flask

Reagents:
- Calcon indicator solution (0.4 % in methanol)
- 0.01 M Titriplex III solution resp. comparable products of other manufacturers (Komplexon III, Idranal III)
- Potassium hydroxide solution 30 %
- All the above solutions are to be stored in plastic containers
- Magnesium sulphate p.a.
- Triethanolamine

Procedure:

- 250 mL Erlenmeyer flask + 100 mL filtrate
- While constantly shaking, the following items have to be added in the following order
  - 5 drops of triethanolamine
  - 0.1 g magnesium sulphate
  - 20 mL potassium hydroxide solution
  - 0.5 mL Calcon indicator solution
- Titrate with Titriplex III solution up to pure blue colouring

Calculation:

1 mL 0.01 Titriplex III solution = 0.4008 mg Ca^{2+}

$$c\,[mg\,kg^{-1}] = \frac{X\,mL \cdot 10 \cdot 0.48008 \cdot 10{,}000\,cm^2 \cdot V\,[L]}{A_{eff}\,[cm^2] \cdot FG\,[kg]}$$

c (mg kg^{-1}):	Concentration of the extracted cation in the filter sheet
X (m L):	Consumption of Titriplex III
Aeff (cm^2):	Effective filter area during determination
FG (kg):	Weight of the dried test sample per m^2
V (L):	Actual rinsing volume during determination

Results: Results to be indicated in mg kg^{-1} rounded off to 1 mg kg^{-1}

2.3.2. AAS flame technique

Equipment: AAS flame technique
100 mL volumetric flask for standard solutions
Pipettes

Reagents: Ca stock solution 1000 mgL-1
5% acetic acid p.a.

Equipment parameters:
Wave length = 422.7 nm
Burner gas = nitrous oxide (N_2O) / acetylene

Procedure:

- Adjustment of the Ca-specific data at the AAS in accordance with the instructions for use of the measuring unit.
- Preparation of at least two standard solutions of different concentration within the measuring range from the stock solution using the 5 % acetic acid as solvent
- Calibration of the AAS in mg L^{-1}
- Determination of the Ca concentration in mg L^{-1} in the filtrate

Calculation:

$$c\,[\text{mg kg}^{-1}] = \frac{c\,[\text{mg L}^{-1}] \cdot 10{,}000\ \text{cm}^2 \cdot V\,[\text{L}]}{A_{\text{eff}}\,[\text{cm}^2] \cdot FG\,[\text{kg}]}$$

c (mg kg^{-1}): Concentration of the extracted cation in the filter sheet
c (mg L^{-1}): Measured value at the cation determination in the filtrate

Aeff (cm²): Effective filter area during determination
FG (kg): Weight of the dried test sample per m²
V (L): Actual rinsing volume during determination

Results: Results to be indicated in mgkg^{-1} rounded off to 1 mg kg^{-1}

2.4. Determination of aluminium

Equipment: AAS flame technique
100 mL volumetric flask for standard solutions
Pipettes

Reagents: Al stock solution 1000 mgL-1
5% acetic acid p.a.

Equipment parameters:
Wave length = 309.3 nm
Burner gas = nitrous oxide (N_2O) / acetylene

Procedure:

- Adjustment of the Al-specific data at the AAS in accordance with the instructions for use of the measuring unit.
- Preparation of at least two standard solutions of different concentration within the measuring range from the stock solution using the 5 % acetic acid as solvent
- Calibration of the AAS in mg L^{-1}
- Determination of the Al concentration in mg L^{-1} in the filtrate

Calculation:

$$c\,[\text{mg kg}^{-1}] = \frac{c\,[\text{mg L}^{-1}] \cdot 10{,}000\ \text{cm}^2 \cdot V\,[\text{L}]}{A_{\text{eff}}\,[\text{cm}^2] \cdot FG\,[\text{kg}]}$$

c (mg kg^{-1}): Concentration of the extracted cation in the filter sheet
c (mg L^{-1}): Measured value at the cation determination in the filtrate
Aeff (cm^2): Effective filter area during determination
FG (kg): Weight of the dried test sample per m^2
V (L): Actual rinsing volume during determination

Results: Results to be indicated in mg kg^{-1} rounded off to 1 mg kg^{-1}

2.5. Determination of heavy metals (As, Cd, Cr, Cu, Ni, Pb)

Depending on the concentration of the cations to be determined in the filtrate produced as under item 2.1. measuring methods of different sensitivity have to be applied.

Equipment: AAS flame technique
Graphite tube technique
MHS technique

Reagents: Standard stock solutions of the cations to be examined
(c = 1000 mgL^{-1})
5 % acetic acid p.a.
Reduction agents: Na(BH4), NaOH (for MHS technique)

Parameters: The equipment parameters are to be taken from the relevant equipment manual.

Procedure:

- Adjustment of the specific data of the respective cation to be examined at the AAS in accordance with the instructions for use of the measuring unit.
- Preparation of at least two standard solutions of different concentration within the measuring range from the stock solution using the 5 % acetic acid as solvent
- Calibration of the AAS in mg L^{-1}
- Determination of the cation concentrations in mgL^{-1} in the filtrate produced as under item 2.1.

Calculation:

$$c\,[mg\,kg^{-1}] = \frac{c\,[mg\,L^{-1}] \cdot 10{,}000\ cm^2 \cdot V\,[L]}{A_{eff}\,[cm^2] \cdot FG\,[kg]}$$

c (mg kg^{-1}): Concentration of the extracted cation in the filter sheet
c (mg L^{-1}): Measured value at the cation determination in the filtrate

Aeff (cm^2): Effective filter area during determination
FG (kg): Weight of the dried test sample per m^2
V (L): Actual rinsing volume during determination

Results: Results to be indicated in mg kg^{-1}.
measured values > 5 mg kg^{-1} rounded off to 1 mg kg^{-1}
measured values < 5 mg kg^{-1} rounded off to 0.1 mg kg^{-1}

3. Mechanical strength

3.1. Wet breaking strength

Purpose: The objective of the tensile test is the determination of the wet breaking strength of wetted samples.

Principle: The wet breaking strength is the stress measured during the tensile test with the wetted sample at the moment of sample breakage (stress at break). To be applied only for hydrophilic depth filter sheets.

Equipment:
- Tensile testing machine (with reference to DIN 51 221 part 1)
  Increase of the stress strength: 5 Ns^{-1}
- Cutting tool
- Wetting device

Preparation of the sample:

- The test sample is cut in machine direction.
- The sample length has to be dimensioned in a way that it can be fixed in the tensile testing machine with a free clamping length of (100 ± 2) mm.
- Width of the test sample: 50 mm.
  The cutting edges of the test samples have to be straight, parallel, smooth and faultless.
  For wetting the samples are put into a receptacle which is large enough that the samples are completely submerged and the water has access to each sample from every side.
  The wetting time is 3 minutes at ambient temperature.

Procedure:

- The test is carried out in accordance with the instructions for use of the measuring unit.

Results: Results to be indicated in N, rounded off to 1 N.

Literature: with reference to DIN 53 112 part 2

4. Filtration characteristics

4.1. Water flow (WD) Wasser-Durchfluß

Purpose: The objective of the test is the determination of the water permeability.

Principle: The water flow is the quotient of the water quantity flowing through the test sample under the conditions of these directions and the duration of flow.

Equipment: Permeameter from Messrs. VEL with a screen as sheet support
Thickness of wire: 0.45 mm
Mesh size: 0.8 mm
Pressure reducing unit with pressure gauge,0 - 250 kPa, class 0.6
Stop watch

Procedure:

- Assembly of the test unit according to the instructions for use.
- Testing area 20 cm^2.
- Medium: tap water of 20 ± 2° C.
- Wetting of the test sample for 3 minutes before it is inserted (conditions analoguous to 3.1.)
- Filling of the chamber on the filtrate side with water.
- Insertion of the test sample, closing of the unit.
- Filling of the unit with water (approx. 550 mL).

1) Test pressure: Δ p = 100 kPa
- 300 mL pre-run at Δ p = 100 kPa
- Determination of the time for the measuring volume of 200 mL (measurement of 300 - 500 mL)

2) Test pressure: Δ p = 20 kPa
- If the flow time for the above described conditions is below 20 s, the test pressure has to be reduced to Δ p = 20 kPa.
- 4 x 500 mL pre-run at Δ p = 100 kPa
- Refill the measuring unit with water

- 300 mL pre-run at Δ p = 20 kPa
- Determination of time for the measuring volume of 200 mL (measurement of 300 - 500 mL)

For the calculation the mean value of the results of three test samples (individual determination) is used.

Calculation:

$$WD\ [L\ m^{-2}\ min^{-1}] = \frac{100{,}000}{t[s] \cdot \Delta\, p\ [kPa]}$$

WD ($Lm^{-2}min^{-1}$): Water flow (Wasser-Durchfluß)
t (s): Time for 200 mL
Δ p (kPa): Pressure

Results: Results to be indicated in $Lm^{-2}\ min^{-1}$ without decimal.

Calibration of the measuring unit:

The calibration of the permeameter of VEL is carried out with corresponding orifice gauges.

- d = 80 mm
- Bottom side plane
- Top side with nut for a gasket ring

(Internal diameter di = 65 mm, size of the gasket d = 3 mm)

- Size of bore: for the test pressure Δ p = 200 kPa 0.3 mm
  for the test pressure Δ p = 50 kPa 0.6 mm
- Material of the orifice gauge: e.g. brass

For the purpose of calibration the screen plate in the measuring unit is removed and the corresponding orifice gauge is centrally positioned above the discharge opening with the gasket ring facing upward. After the test unit has been assembled it is filled with 300 mL of demineralized water, prefiltered via an 0.45 µm membrane. The time is determined for the flow of 100 mL (e.g. of 100 - 200 mL) as 5-fold determination.

Source: With reference to Analytica EBC, 4th edition 1987,
page D 257/11.8. Filtration output, EBC Routine Method

4.2. Titer reduction

Purpose: Determination of the bacteria retention of depth filter sheets under the conditions described in these directions.

Principle: The depth filter sheet is challenged with defined quantities of the test organism, subsequently the organism counts are determined in the respective filtrates.

4.2.1 Method 1

The present directions for the determination of the titer reduction are suitable for a direct comparison of filter sheets. Assignments of LRV values over longer periods and from different laboratories are not possible because of the variety of external influences (e.g. variations in the different lots of nutrients).

Equipment:
- Holding vessel for buffer
- Positive-displacement pump
  (e.g. Netzsch Mohno pump type 4NU04)
- Flow meter (measuring range 0.5 - 2 Lh-1)
- Single sheet filter, d = 6 cm; Aeff: 20 cm²
- Membrane filter
- Suction device
- Stop watch
- 1 mL syringe
- Autoclave
- General laboratory equipment

Nutrients:
- Tryptone soya agar (TSA)
  acc. to the European Pharmacopeia 2 or USP XXIII

Pancreatic digest of casein	15.0 g
Papaic digest of soybean meal	5.0 g
Sodium chloride	5.0 g
Agar	15.0 g
Water (deionized)	1000 mL
pH-value	7.3 ± 0.2 after sterilization

- Tryptone soya broth (TSB)
  (for B. diminuta and Serr. marcescens)

Pancreatic digest of casein	17.0 g
Papaic digest of soybean meal	3.0 g
Sodium chloride	5.0 g
Di-basic potassium phosphate	2.5 g
D(+) glucose	2.5 g
Water (deionized)	1000 mL
pH-value	7.3 ± 0.2 after sterilization

Diluent: - Sodium chloride solution
9 g sodium chloride, 1000 mL water (deionized)
pH value 7.2 ± 0.1

Filtration medium:
The filtration medium shall be a sterile phosphate buffer (according to Sörensen) freshly prepared on the day of the test:

Di-sodium hydrogen phosphate dihydrate	7.26 g
Potassium hydrogen phosphate	3.52 g
Water (deionized)	1000 mL
pH value	7.0

Test organisms:
- Brevundimonas diminuta ATCC 19 146
- Serratia marcescens ATCC 14 756

Storage of the stock cultures according to DIN 58941 part 2.
Streaked plate of the stock culture on TSA

Incubation: B. diminuta 48 - 72 h at (30 ± 2)° C
Serr. marcescens 24 - 48 h at (30 ± 2)° C
The incubation is carried out until single colonies can be distinctly identified.

Preculture: Inoculation of 10 mL TSB with a single colony of the streaked plate on TSA.
Incubation: B. diminuta approx. 24 h at (30 ± 2)° C
Serr. marcescens approx. 24 h at (30 ± 2)° C

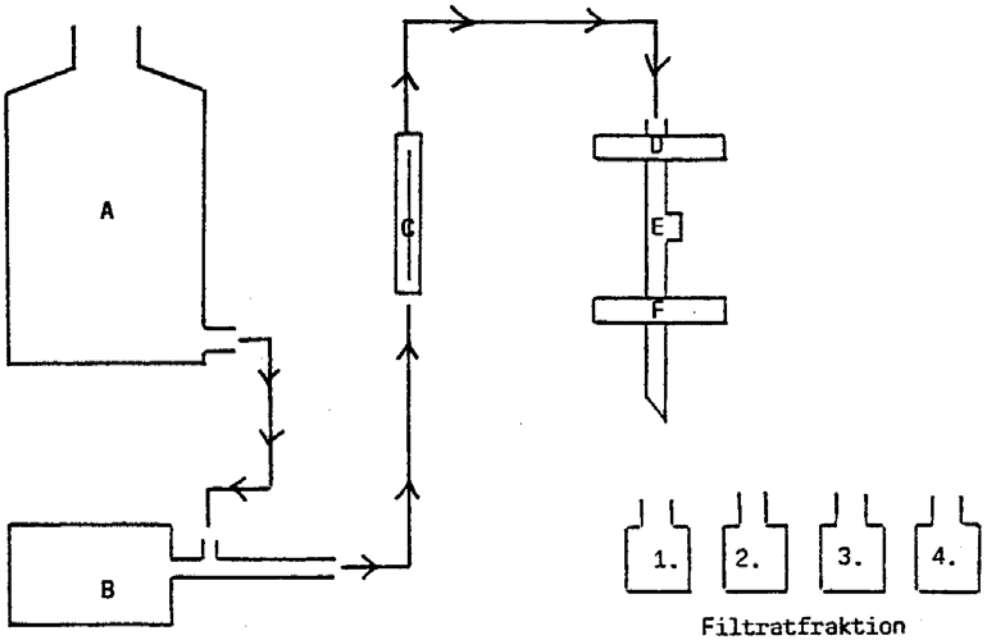
Main culture: For the test 200 mL of nutrient solution are inoculated with 0.5 mL of the preculture and incubated under defined conditions at (30 ± 1)° C on the magnetic stirrer. Prior to the test the main cultures have to be microscopically monitored for purity and for the presence of single cells.

The viable organism count of the main culture has to be at least 2 x 10^9 CFU mL^{-1} in order to achieve a bacteria challenge of 10^7 CFU per cm^2 filter area in the 3rd fraction of the test.

Preparation of the dilutions for bacteria dosage

- Main culture, undiluted 1 mL dosed for 4th fraction
- Main culture, diluted 1:10 1 mL dosed for 3rd fraction
- Main culture, diluted 1:100 1 mL dosed for 2nd fraction
- Main culture, diluted 1:1000 1 mL dosed for 1st fraction

Test stand:



A = Holding vessel **C = Flow meter** **E = T-piece for bacteria dosage**
B = Displacement pump **D = Membrane filter** **F = Sheet filter**

Test procedure:

- To start the test the cleaned, non-sterile unit is installed (see sketch of test stand).
- The actual test unit (membrane filter holder, T-piece for bacteria dosage, sheet filter with inserted test sample) is autoclaved for 20 min. at 121° C.
- Moreover all the required glass vessels and all the necessary parts for membrane filtration of the filtrates are autoclaved.
- After autoclaving a pre-sterile 0.2 µm mixed ester membrane is inserted under sterile conditions into the membrane filter holder.
- The filtration medium is pumped to the test unit by means of a positive-displacement pump with a volume flow of V = (500 ± 50) L $m^{-2}h^{-1}$.
- In the membrane filter the filtration medium is sterile-filtered and flows across the filter sheet to be tested.
- The filter sheet is pre-rinsed with 250 mL. During rinsing of the sheet the filtration velocity is corrected to the rated output, if necessary.
- Without interrupting the filtration the 1:1000 dilution of the main culture (= lowest organism count) is added after rinsing.
- The dosage of the test organism is carried out with a 1 mL syringe within 20 s at the beginning of each fraction.
- Filtrate volume: 100 mL to be collected under sterile conditions.
- Subsequently the three increasing concentrations (1:100, 1:10, undiluted main culture) are added according to the same principle.
- During filtration the filtration velocity is not changed.
- To determine the organism count of the filtrates 0.1 mL, 1 mL and the rest of the filtrate are membrane-filtered. 0.1 mL and 1 mL are diluted by 50 mL of sterile buffer before membrane filtration.
- For the determination of the bacteria challenge of the filter sheet the viable organism count of the main culture is determined by plate counting.
- The plates from the filtrate samples and from this organism count are commonly incubated and evaluated.

Incubation temperature:	B. diminuta	30° C
	Serr. marcescens	30° C
Incubation time:	B. diminuta	48 h
	Serr. marcescens	24 h

Note:

When using a new, freeze-dried preparation of the corresponding test organism and when using a new lot of nutrients, the behaviour of the test organisms has to be investigated using standard sheets with a determined bacteria challenge.

Evaluation:

After incubation, the evaluation is carried out by counting the colonies on the plates from the organism count of the main culture as well as the control membranes from the filtrate. The results are to be documented.

- The number of organisms of the main culture and of the dilutions which were dosed is then calculated and indicated in CFU mL^{-1}.

- The calculation of the specific bacteria challenge in CFU/cm² is carried out for every fraction.

$$\text{Spec. bacteria challenge} = \frac{\text{Number of bacteria of the dosed suspension (CFU/mL) x dosed volume [mL]}}{\text{effective filter area (cm²)}}$$

The specific bacteria challenges are added from fractions 1 to 4.

For every fraction a LRV is calculated (LRV = log reduction value)

$$\text{LRV} = \log \frac{\text{Total CFU in the unfiltrate}}{\text{Total CFU in the filtrate of the fraction}}$$

In case of a sterile filtrate CFU is put at 1.0, as a division by 0 is not defined.

From the 4 LRV values of the fractions the arithmetical mean, the so-called mean LRV, is calculated which is the characteristic size for the bacteria retention capability of the depth filter sheet. If several filtrates are sterile, only the last value - corresponding to the highest challenge - is taken for the calculation.

Results: Results to be indicated in LRV values, rounded off to 0.1.

Literature: with reference to DIN 58 355 part 3.

4.2.2 Method 2

Equipment:
- 4 glass bottles (1 L) with 1 L of sterile phosphate buffer
- hose pump (or similar)
- when using strongly pulsating pumps it is recommendable to use buffer vessels to reduce the pulsation strength
- holding vessel with sterile phosphate buffer
- single sheet filter, d = 6 cm; Aeff: 20 cm²

Nutrients, diluent, test organisms, filtration medium, preculture, stock culture: see under test methods for filter sheets ('5 93)

Preparation of the main culture:
In a 300 mL culture flask with 3 baffles, 150 mL of nutrient solution are inoculated with 0.5 mL of preculture and agited at 30°C in the shaking incubator at 130 rpm. The incubation time has to be such that at the day of the test an optical density of 7 at 578 nm is not exceeded.

Preparation of the bacteria suspensions (for ten individual determinations):

1. 1 L- bottle with sterile phosphate buffer: add 10 mL of main culture, diluted 1 : 1000
2. 1 L- bottle with sterile phosphate buffer: add 10 mL of main culture, diluted 1 : 100
3. 1 L- bottle with sterile phosphate buffer: add 10 mL of main culture, diluted 1 : 10
4. 1 L- bottle with sterile phosphate buffer: add 10 mL of main culture, undiluted.

100 mL of this bacteria suspension thus have the same organism count as the corresponding 1 mL fractions according to method 1 (as under 4.2.1.).
100 mL of each bacteria suspension are thus necessary per test sample with a diameter of 6 cm (i.e. 200 mL in case of double determination etc.)

Test procedure:

- Before starting the test, all parts getting in contact with the organisms are autoclaved for 20 min at 121°C (pumping hoses, filter holder with the wetted test sample, filtrate collecting vessels etc.)
- By means of a pump, the sterile buffer is pumped through the plant at a volume flow of 500 L m^{-2} h^{-1}
- The test sample is pre-rinsed with 250 mL of sterile buffer.
- Shortly before rinsing is finished the filtrate collecting vessel is fitted to the filtrate outlet, possibly under sterile conditions.
- Without interrupting filtration, the pump suction hose is put into the vessel with the lowest organism count until 100 mL of the bacteria suspension have been drawn off. After that the hose is put back into the vessel with sterile buffer - also without interrupting the fitration
- In order to ensure that the 100 mL of bacteria suspension have passed through the filter, 200 mL filtrate or more have to be collected - depending on the dead volume of
  the plant (the bacteria challenge does not change thereby).
- Subsequently the filtrate collecting vessel is replaced by a new one and the hose is put into the vessel with the next higher bacteria count (This procedure has to be repeated for all fractions)
- After the test the organism count in the filtrate and the viable organism count of the main culture are determined as per 4.2.1.

Evaluation and calculation are also carried out as per 4.2.1.

Literature: with reference to DIN 58 355 part 3.

4.3. Clarifying effect

4.3.1. Adsorption

Purpose: Determination of the adsorptive characteristics of depth filter sheets.

Principle: The depth filter sheet is challenged with a defined colour solution with negative charge; the decrease of the colour concentration is determined.

Equipment: Single sheet filter, d = 6 cm; Aeff: 20 cm²
Hose pump
Photometer, λ = 505 nm

Solution: Azorubin red, 40 mgL-1,
(e.g. Serva Feinbiochemica GmbH &Co.,ordering No.: 14410)

Filtration parameters:

A_{eff} = 20 cm^2
V = 1,000 L m^{-2} h^{-1} = 33.3 mL min^{-1}
(shortly 3,000 L m^{-2} h^{-1} = 100 mL min^{-1})

Procedure:

- Install the filter holder horizontally.
- Starting quantity: 200 mL azorubin red solution.
- Fill the lower part of the filter holder up to the edge with the colour solution.
- Put the dry test sample in the filter holder in a way that no air bubble is enclosed.
- Assemble the filter holder.
- Filtration of the solution in circuit at V ~ 33.3 mL min^{-1}.
- After approx. 5 minutes shortly increase the volume flow (for 15 - 30 s) to 100 mL min^{-1} in order to press the remaining air out of the test sample.
- When colour is no longer adsorbed from the solution (~ 20 min.) the extinction is determined.
- If the extinction is < 0.1 a defined quantity has to be added to the test and filtration in circuit is continued.

Calculation:

$$\frac{(E_A - E_F) \cdot C_A\,[g\,L^{-1}] \cdot V_A[L] \cdot 10{,}000\,cm^2}{E_A \cdot 20\,cm^2} = \text{Adsorption}\,[cm]$$

E_A: Extinction of the starting solution
E_F: Extinction of the filtrate
C_A: Concentration of the azorubin red solution (0.04 g L^{-1})
V_A: Volume of the starting solution (L)

For 0.2 L of starting solution:

$$\frac{(E_A - E_F) \cdot 4}{E_A} = \text{Adsorption}\,[g\,m^{-2}]$$

Results: Indication of the results:

0 - 1 g m^{-2}	*no adsorption*
>1 - 2 g m^{-2}	*poor adsorption*
>2 - 4 g m^{-2}	*moderate adsorption*
>4 gm^{-2}	*high adsorption*

4.3.2. Mechanical clarifying effect

Purpose: Determination of the retention capability of the depth filter sheet with regard to turbid particles of a model solution.

Principle: The depth filter sheet is challenged with the model suspension under defined conditions and the filtration behaviour is determined (output and turbidity in the filtrate).

Note:

With this test to examine the mechanical clarifying effect of a depth filter sheet absolute figures cannot be determined as on the one hand the necessary reproducibility in preparing the model suspension is not given and on the other hand particle size distribution changes in the course of time so that even in repeat tests identical figures cannot be obtained. With this test it is only possible to carry out parallel filtrations. The best reproducibility of results is achieved if filtration starts between 0.5 to 3.5 hours after preparation of the model suspension.
As the substances of the model suspension are food products it has to be ensured that the complete filtration unit is disinfected in adequate intervals.

Equipment:
- Filtration unit with several uniform filter holders (Aeff > 100 cm²) and holding vessel
- Balance
- Turbidity photometer (e.g. Sigrist KTN)

Model suspension:
- Ovomaltine, WASA GmbH, Celle
- Coffee substitute extract, cyclone product; ingredients: barley, rye, chicory, malt. Producer: Günzburger Nahrungsmittelwerke, Günzburg

Preparation for 100 L:
- Add (by stirring) 7.0 g coffee substitute extract and 2.0 g Ovomaltine to about 1 L of water (20° C) and fill this mixture into the prefilled holding vessel
- Top up the holding vessel to 100 L and homogenize the suspension.
- If the suspension is not used-up after 4 hours it has to be discarded.

Procedure:

- Insert the test samples into the filter holder and rinse with water for 5 minutes (sterilizing sheets have to be rinsed with a Δp of > 150 kPa, all other depth filter sheets with a Δp of > 100kPa).
- Subsequent filtration of the model suspension.
- The filtration parameters have to be adapted to the test sample and the test unit.

  Method A: (Δp = constant): Determination of the filtrate volume after a fixed time resp. up to blockage of the test sample.

  Method B: (V = constant): Determination of the Δp after a fixed time resp. of the time with a fixed max. Δp.

- Determination of the turbidity after fixed time intervals.

Indication of the results:

a) Mechanical clarifying effect - turbidity (MKT)
b) Mechanical clarifying effect - volume (MKV), related to 100 cm^2

Evaluation:

Comparative evaluation of the test samples from one filtration run.

5. Sensory test (odour and taste)

Purpose: This method gives information about possible influences of the depth filter sheet on odour or taste.

Principle: The depth filter sheet is rinsed with still mineral water, and the filtrate is sensorially tested.

Equipment: Laboratory filter d = 14 cm with a filling space of 2 L.

Reagents: 2 L of still mineral water.

Procedure:

A test sample with a diameter of 14 cm, corresponding to about 130 cm^2 filter area is pre-rinsed with 1.3 L of still mineral water. For the sensory test the following 0.5 L are used. The water temperature is 80° C. This finally filtered water is tested in comparison to the unfiltered water (blank value) after cooling down to room temperature.
V = 500 ± 50 L $m^{-2}h^{-1}$

Evaluation:

The results are indicated by relating a certain sensory impression to a certain mark. The graduation of the marks ranges from 0 - 3.

The test should be carried out by at least 3 experienced testers.

Tester 1	**Tester 2**	**Tester 3**
Mark:	Mark:	Mark:

Graduation of marks: 0 = no deviation
1 = minor deviation
2 = distinct deviation
3 = strong deviation