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Stationary source emissions — Bioaerosols and biological agents — Sampling of bioaerosols and collection in liquids — Impingement method

National foreword

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European foreword

This document (EN 17359:2020) has been prepared by Technical Committee CEN/TC 264 “Air quality”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by February 2021, and conflicting national standards shall be withdrawn at the latest by February 2021.

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1 Scope

This document contains specifications for active sampling of bioaerosols from exhaust air flowing through a defined cross-section of a stack. It defines general principles that have to be taken into account during an isokinetic sampling campaign for bioaerosols by bubbling the exhaust air through a specific impinger designed for emission measurements.

In this document the application with culturable organisms is specified but the same principle might be applicable for non-cultural based methods (e.g. molecular and/or enzyme-based methods).

The impinger is designed to allow a sample volume flow of 1 m³/h to 1,8 m³/h, or 16 l/min to 30 l/min, respectively, and has been tested with regard to various microorganisms within broad concentration ranges [1; 2; 3; 4].¹

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 1040:2005, *Chemical disinfectants and antiseptics - Quantitative suspension test for the evaluation of basic bactericidal activity of chemical disinfectants and antiseptics - Test method and requirements (phase 1)*

EN 13284-1:2017, *Stationary source emissions - Determination of low range mass concentration of dust - Part 1: Manual gravimetric method*

EN 15259:2007, *Air quality - Measurement of stationary source emissions - Requirements for measurement sections and sites and for the measurement objective, plan and report*

CEN/TS 16115-1, *Ambient air quality - Measurement of bioaerosols - Part 1: Determination of moulds using filter sampling systems and culture-based analyses*

EN ISO 16911-1, *Stationary source emissions - Manual and automatic determination of velocity and volume flow rate in ducts - Part 1: Manual reference method (ISO 16911-1)*

EN ISO 20988:2007, *Air quality - Guidelines for estimating measurement uncertainty (ISO 20988:2007)*

¹ This method is accepted by convention as reference method for determination of total emissions under application of an out stack configuration according to EN 13284-1.

3 Terms and definitions

For the purposes of this document, the following terms and definitions.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- ISO Online browsing platform: available at <https://www.iso.org/obp>
- IEC Electropedia: available at <http://www.electropedia.org/>

3.1

waste gas

exhaust air

carrier gases with solid, liquid or gaseous constituents (emissions)

Note 1 to entry: The carrier gases can be natural air (e.g. from stable ventilation) or process gases.

3.2

analytical blank value

value determined by a blank sample covering the analytical procedure to ensure that no significant contamination occurs during the complete analytical procedure

Note 1 to entry: according to CEN/TS 16115-1

3.3

bacteria

large group of prokaryotic microorganisms with one chromosome in a nuclear region and which replicate only asexually by cell division

[SOURCE: EN 13098:2019, 3.3]

Note 1 to entry: The current classification of bacteria, i.e. the grouping to genera and species, is done according to molecular sequence-based, chemotaxonomic and physiological properties.

3.4

standard conditions

reference values for a dry gas at a pressure of 101,325 kPa rounded to 101,3 kPa and a temperature of 273,15 K rounded to 273 K

Note 1 to entry: Where a regulatory authority stipulates reference conditions, for example through a site Licence or Permit, this shall override the conditions specified above.

3.5

bioaerosol-

airborne particles of biological origin

[SOURCE: EN 13098:2019, 3.1]

Note 1 to entry: Bioaerosols in the sense of this European Standard are all aggregations of particles in the atmosphere to which fungi (spores, conidia, fragments of hyphae), bacteria, viruses and/or pollen as well as their cell membrane components and metabolites (e.g. endotoxins, mycotoxins) are attached or that consist of the above mentioned components.

3.6

biological sampling efficiency

capacity of the sampler to maintain the viability of the airborne microorganisms during collection and also to keep the microbial products intact

[SOURCE: EN 13098:2019, 3.4]

Note 1 to entry: The biological sampling efficiency considers the sampling stress occurring during sampling and analysis in addition to the physical sampling efficiency. It is strain- and species specific.

3.7

endotoxin

constituent of the external membrane of Gram-negative bacteria (lipopolysaccharide), consisting of a complex lipid, lipid A, which is covalently bound to a polysaccharide

Note 1 to entry: "Free endotoxin" is liberated after cell death and by budding from living cells. Lipid A is the active (toxic) part and is a potent pro-inflammatory substance and may induce febrile, bronchial and other symptoms in exposed workers. The composition and the toxicity of endotoxin differs between species.

[SOURCE: EN 13098:2019, 3.9]

3.8

load

product of measured concentration of bioaerosols and the volumetric flow rate of the stack of the plant

3.9

overall blank sample

field blank sample

sample taken at the plant site in an identical manner to the normal samples in the series, except that the sampling system is not inserted into the duct and no gas is sampled during test duration

Note 1 to entry: In this standard the term field blank sample is used.

3.10

total cell count

total number of viable and dead cells in a given volume

Note 1 to entry: The total cell count can be determined e.g. by DAPI staining.

Note 2 to entry: When for example stained with DNA fluorescent dyes like DAPI (4,6-Diamidino-2-phenylindole-dihydrochloride), living and dead cells are jointly counted and cannot be distinguished.

3.11

main volume flow

volumetric flow of the flue gas in the sampling plane

3.12

impingement

separation of airborne particles in liquids by different mechanisms, i.e. impaction, diffusion, interception and sedimentation

3.13**Colony Forming Unit****CFU**

unit by which the culturable number of microorganisms is expressed

[SOURCE: EN 13098:2019, 3.6 and 3.7]

Note 1 to entry: One Colony Forming Unit can originate from one single microorganism, an aggregate of many microorganisms or from one or many microorganisms attached to one particle.

Note 2 to entry: The number of colonies which develop depends on cultivation conditions.

3.14**culture based analyses****cultivation**

growing of microorganisms on culture media

[SOURCE: ISO 16000-16:2008, 3.6]

3.15**non-culture-based analyses**

methods which do not rely on cultural methods including: microscopy, molecular methods, cytometry, fluorescence etc.

3.16**indicator organisms**

microorganisms which are characteristic for the emissions of a specific type of plant and can be detected with currently available methods of sampling and analysis

Note 1 to entry: Indicator organisms which are characteristic of a specific source (process) can also occur in air not influenced by the respective source, but mostly in minor concentrations. This is explained by the fact that many microorganisms are found ubiquitously.

3.17**sampling line****measurement line**

line in the sampling plane along which the sampling points are located, bounded by the inner duct wall

[SOURCE: EN 15259:2007, 3.15]

3.18**measurement site**

place on the flue gas duct in the area of the sampling plane consisting of structures and technical equipment, for example measurement platforms, measurement ports and energy supply

[SOURCE: EN 15259:2007, 3.11 – modified]

3.19**sampling point****measurement point**

specific position on a sampling line at which a sample is extracted or the measurand directly determined

[SOURCE: EN 15259:2007, 3.16]

3.20

sampling plane measurement plane

plane normal to the centreline of the duct at the sampling position

[SOURCE: EN 15259:2007, 3.13]

Note 1 to entry: In the case of emission measurements of area sources, the total surface corresponds to the sampling plane.

3.21

microbial air pollution

concentrations of airborne microorganisms that exceed natural concentrations or compositions which differ in type from those occurring naturally

3.22

microorganism

microbial entity, either cellular or non cellular, that is capable of multiplication or transfer of genetic material, or entities that have lost these properties

[SOURCE: EN 13098:2019, 3.18]

3.23

physical sampling efficiency

capacity of the sampling device to collect particles suspended in air

[SOURCE: EN 13098:2019, 3.20]

3.24

fungi

unicellular yeasts and filamentous fungi from several taxonomic groups namely zygomycetes (Mucormycotina and Entomophthoromycotina), ascomycetes (Ascomycota) and mitosporic fungi (deuteromycetes, fungi imperfecti) [7]

Note 1 to entry: Moulds form different types of spores depending on the taxonomic group they belong to, namely conidiospores (conidia), sporangiospores or ascospores.

3.25

sample volumetric flow

volumetric flow taken off from the main stream for determination of the measured component

[SOURCE: EN 15259:2007, 3.29]

3.26

sampling train

fully assembled sampling system as per Figure 1

4 Symbols and abbreviations

The symbols as outlined below are used throughout this standard:

Symbol	Unit	Meaning
A	m^2	sampling plane of the duct
A_S	m^2	area calculated on the basis of the diameter δ_S of the nozzle
c	$\text{CFU}/\text{m}^3; \text{TCC}/\text{m}^3$	number concentration of microorganisms in the sample flow
C	$\text{CFU}/\text{m}^3; \text{TCC}/\text{m}^3$	number concentration of microorganisms in the exhaust air
ΣC_{plate}	CFU	sum of counted colonies on all evaluated plates
d		dilution factor of the first evaluated dilution (for example $d = 100$ at a dilution degree 10^{-2})
D		additional dilution factor, which results from the fact that only one aliquot of the dilutions has been outplated
f_{GM}	kg/m^3	sample gas volume humidity at the volume meter (relative to the standard conditions)
f	kg/m^3	humidity in the exhaust air
n_1		number of plates at the lowest evaluable dilution (higher concentration)
n_2		number of plates at the nearest higher evaluable dilution (lower concentration)
N		number of duplicate analyses
N_{CFU}	CFU	number of bacteria in cfu
p		pipetting factor
p_d	Pa	dynamic pressure
p_G	kPa	absolute exhaust air pressure
p_{GM}	kPa	absolute pressure at the gas volume meter
p_N	kPa	standard pressure (101,3 kPa)
p_A	kPa	ambient air pressure
p_R	kPa	absolute pressure at the float-type flow meter inlet
p_{St}	kPa	static pressure in the duct
$r_{\text{H}_2\text{O}, G}$	%	volume fraction of water vapour in the humid exhaust air
$r_{\text{H}_2\text{O}, \text{GM}}$	%	volume fraction of water vapour in the humid exhaust air at the gas volume meter
s	$\text{CFU}/\text{m}^3; \text{TCC}/\text{m}^3$	standard deviation under repeatability conditions
T_G	K	absolute exhaust air temperature

Symbol	Unit	Meaning
T_{GM}	K	absolute temperature at the gas volume meter
T_N	K	standard temperature (273 K)
T_R	K	temperature at the float-type flow meter inlet
$u_{G,i}$	m/s	flow velocity at the measurement point i
$u_{average}$	m/s	average flow velocity within the measurement section
u_S	m/s	flow velocity in the entry nozzle
u		uncertainty
v	m ³	sample gas volume
v_b	ml	volume of pipetted aliquot
v_{GM}	m ³	sample gas volume relative to the condition at the gas volume meter (temperature, pressure, humidity)
$v_{N,tr}$	m ³	sample gas volume (relative to the standard conditions 273 K, 101,3 kPa and dry gas volume)
v_p	ml	volume of pipetting (plates 1-4)
v_1	ml	filtered volume in the impinger sampling liquid
v_2	ml	total volume of the impinger sampling liquid
$\dot{v}$	m ³ /h	sample volume flow (respectively, the envisaged sample gas volume in the impinger)
$\dot{v}_{N,tr}$	m ³ /h	sample volumetric flow (relative to the standard conditions 273 K, 101,3 kPa and dry gas volume)
$\dot{v}_i$	m ³ /h	required sample gas volume at measurement point i relative to the exhaust air conditions (temperature, pressure, if applicable: humidity)
$\dot{v}_{i,N,tr}$	m ³ /h	required sample gas volume at measurement point i (relative to the standard conditions 273 K, 101,3 kPa and dry gas volume)
$\dot{V}$	m ³ /h	main volume flow of the exhaust air relative to the exhaust air conditions (temperature, pressure, if applicable: humidity)
$\dot{V}_N$	m ³ /h	main volume flow of the exhaust air (relative to the standard conditions 273 K, 101,3 kPa and dry gas volume)
x_{1i}	CFU/m ³ ; TCC/m ³	measured value in impinger 1 of the test series i
x_{2i}	CFU/m ³ ; TCC/m ³	measured value in impinger 2 of the test series i
$z_{Impinger}$	TCC	determined cell count in the evaluated impinger
z_{TCC}	TCC	count of the microorganism cells in the sample
z_{MO}	CFU	count of the microorganisms in the sample

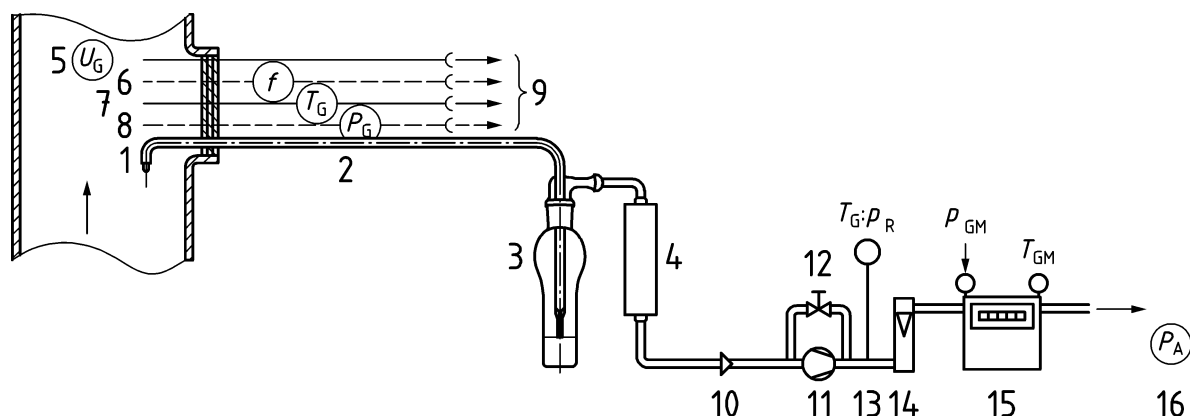
Symbol	Unit	Meaning
$\dot{Z}_{MO}$	CFU/h	load of the culturable microorganisms (relative to the count) in the main gas volume of the exhaust air
$\dot{Z}_{TCC}$	TCC/h	load of the microorganism cells (relative to the count) in the main gas volume of the exhaust air
δ_S	m	diameter of the entry nozzle
ρ_f	kg/m ³	density of the humid exhaust air
ρ_{H_2O}	kg/m ³	density of the gaseous water (relative to the standard conditions, $\rho_{H_2O} = 0,804 \text{ kg/m}^3$)
ρ_N	kg/m ³	standard density of exhaust air
τ	s	sampling duration

5 Principle of method

The method for measurement of bioaerosol emissions described below is based on EN 13284-1. The method describes the procedure of emission measurements under isokinetic conditions using an emission impinger in an out of stack configuration.

For sources where exhaust air flows through a defined cross-section, a sample volume flow of the exhaust air is extracted from the main volume flow for the duration of sampling and the sample gas volume is measured. The number and position of the representative measurement points is determined in EN 15259, although the maximum number of points to be used for a single test will be limited to 8 (see 7.3.1). As per EN 13284-1, the isokinetic rate shall be maintained between -5 % and +15 %.

When performing bioaerosol emission measurements, exhaust air is passed through the entry nozzle and sampling probe before the particles suspended in the exhaust air are captured in the impinger sampling solution. It is known that particles can be deposited in bends and on the surface of the probe, therefore, rinsing of all of the parts of the sampling train upstream of the emission impinger is required after sampling.



Key

- 1 entry nozzle with a bend
- 2 sampling probe
- 3 emission impinger (it is possible to use more than one impinger, see chapter 8.3.1)
- 4 drying system (for example silica gel) humidity separator
- 5 device for exhaust air velocity measurement (hot-wire anemometer, fan-wheel anemometer, pitot tube)
- 6 where appropriate, humidity sensing device
- 7 temperature sensing device
- 8 where appropriate, sensor for absolute pressure
- 9 readings recorder
- 10 shut-off valve
- 11 suction unit
- 12 bypass valve
- 13 temperature sensor (T_R) and pressure sensor (p_R)
- 14 float-type flow meter (optional)
- 15 gas volume meter with temperature sensor (T_{GM}) and pressure sensor (p_{GM})
- 16 pressure meter

Figure 1 — Example of a sampling train for bioaerosol measurements

6 Theoretical fundamentals

6.1 Isokinetic sampling

The objective of the isokinetic velocity sampling is the representative measurement of the particles dispersed in a gas flow irrespective of their size. For the ideal case of a directed flow, in the duct during isokinetic sampling are present isokinetic conditions relative to the trajectories of the particles in the free flow and the trajectories in the nozzle. The theoretic basis of the isokinetic sampling is described in detail in Annex A.

6.2 Determination of concentration and load of the microorganisms

The formulae listed below are in principle of general validity; however, they refer here mainly to the number concentration of microorganisms as a component of the bioaerosols. For example, the calculation of the endotoxins can be made in an analogous manner.

The number concentration C of microorganisms in the exhaust air is defined by the ratio of the load to the total volume flow (main volume flow) of the exhaust air:

$$C = \frac{\dot{Z}_{MO}}{\dot{V}} \quad \text{bzw.} \quad C = \frac{\dot{Z}_{TCC}}{\dot{V}} \quad (1)$$

where

$\dot{Z}_{MO}$ is the load of the culturable microorganisms (relative to the number) in the main volume flow of the exhaust air, in CFU/h

$\dot{Z}_{TCC}$ is the load of the microorganism cells (relative to the number) in the main volume flow of the exhaust air, in TCC/h

$\dot{V}$ is the main volume flow of the exhaust air, in m³/h

The load in the exhaust air flow cannot be measured directly. A sample flow abstraction is required for its determination. The sample gas volume v is measured by means of a gas volume meter, or is calculated from the sucked gas volume measured for example by means of a calibrated orifice or a fan-wheel anemometer and the sample duration:

$$v = \int \dot{v}(\tau) d\tau \quad (2)$$

where

v is the sample gas volume, in m³

$\dot{v}$ is the sample volume flow, in m³/h

τ is the sampling duration, in s

The number concentration of the microorganisms in the sample gas volume sucked for the duration of sample results from (see also 10.4):

$$c = \frac{Z_{MO}}{v} \quad \text{bzw.} \quad c = \frac{Z_{TCC}}{v} \quad (3)$$

where

c is the number concentration of the microorganisms in the sample flow, in CFU/m³ or TCC/m³

Z_{MO} is the count of the microorganisms in the sample, in CFU

Z_{TCC} is the count of the microorganisms in the sample, in TCC

v is the sample gas volume, in m³

The load $\dot{Z}_{MO}$ or respectively $\dot{Z}_{GZZ}$ results from the number concentration of the microorganisms c in the sample flow and the volume flow $\dot{V}$ as follows (see also 10.5):

$$\dot{Z}_{MO} = c \cdot \dot{V} \quad \text{bzw.} \quad \dot{Z}_{TCC} = c \cdot \dot{V} \quad (4)$$

where

- $\dot{Z}_{MO}$ is the load of the culturable microorganisms (relative to the number) in the main volume flow of the exhaust air in CFU/h
- $\dot{Z}_{TCC}$ is the load of the microorganism cells (relative to the number) in the main volume flow of the exhaust air in TCC/h
- c is the number concentration of the microorganisms in the sample flow in CFU/m³ or TCC/m³
- $\dot{V}$ is the main volume flow of the exhaust air in m³/h

7 Device and materials

7.1 General

The determination of the load $\dot{Z}_{MO}$ in the exhaust air according to Formula (4) and Formula (15) requires:

- the measurement of the exhaust air parameters for calculation of the main volume flow (see 6.1 and 7.2),
- the sampling of the bioaerosols by means of the isokinetic sample flow separation with measurement of the sample gas volume (see 6.2 and 7.3),
- the subsequent analysis, and
- the calculation of the microorganisms concentration (see 10.4).

7.2 Device and methods for measurement of the exhaust air parameters for the calculation of the main volume flow

7.2.1 General

For a proper application of the measurement method, for standardization of the obtained measurement results and for determination of the load it is necessary to determine the measurands characterizing the exhaust air in the run-up and in the course of the measurement. As a rule these measurands are:

- the exhaust air velocity,
- the air pressure,
- where appropriate, the absolute air pressure of the exhaust air,
- the exhaust air temperature,
- the exhaust air humidity (for calculation of the exhaust air density, as well as for standardization).

7.2.2 Device for determination of the exhaust air velocity

Common devices for determination of the exhaust air velocity are:

- hot-wire anemometer,
- fan-wheel anemometer,
- pitot tube with manometer.