SLOVENSKI STANDARD
SIST EN 17714:2025

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Rastlinski biostimulanti - Določanje koncentracije mikroorganizmov

Plant biostimulants - Determination of microorganisms' concentration

Pflanzen-Biostimulanzien - Bestimmung der Konzentration von Mikroorganismen

Biostimulants des végétaux - Détermination de la concentration en microorganismes

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65.080	Gnojila	Fertilizers

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Plant biostimulants - Determination of microorganisms' concentration

Biostimulants des végétaux - Détermination de la concentration en microorganismes

Pflanzen-Biostimulanzien - Bestimmung der Konzentration von Mikroorganismen

This European Standard was approved by CEN on 26 August 2024.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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European foreword

This document (EN 17714:2024) has been prepared by Technical Committee CEN/TC 455 “Plant biostimulants”, the secretariat of which is held by AFNOR.

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of an identical text or by endorsement, at the latest by May 2025, and conflicting national standards shall be withdrawn at the latest by May 2025.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes CEN/TS 17714:2022.

EN 17714:2024 includes the following significant technical changes with respect to CEN/TS 17714:2022:

— the European foreword and the Introduction have been updated;

— normative references have been updated;

— Annex ZA has been added.

This document has been prepared under a standardization request addressed to CEN by the European Commission. The Standing Committee of the EFTA States subsequently approves these requests for its Member States.

For the relationship with EU Legislation, see informative Annex ZA, which is an integral part of this document.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Türkiye and the United Kingdom.

Introduction

The European Committee for Standardization (CEN) was requested by the European Commission (EC) to draft European Standards or European Standardization deliverables to support the implementation of Regulation (EU) 2019/1009 of 5 June 2019 [1] laying down rules on the making available on the market of EU fertilising products ("FPR" or "Fertilising Products Regulation").

This standardization request, presented as SR M/564 and relevant amendments, also contributes to the Communication on "Innovating for Sustainable Growth: A Bio economy for Europe". The interest in plant biostimulants has increased significantly in Europe as a valuable tool to use in agriculture. Standardization was identified as having an important role in order to promote the use of biostimulants. The work of CEN/TC 455 seeks to improve the reliability of the supply chain, thereby improving the confidence of farmers, industry, and consumers in biostimulants, and will promote and support commercialisation of the European biostimulant industry.

WARNING — Persons using this document should be familiar with normal laboratory practice. This document does not purport to address all of the safety problems, if any, associated with its use. It is the responsibility of the user to establish appropriate safety and health practices and to ensure compliance with any national regulatory conditions.

IMPORTANT — It is absolutely essential that tests conducted in accordance with this document be carried out by suitably trained staff.

1 Scope

This document specifies the general rules to determine the concentration of microorganisms present in plant biostimulants expressed as the number of active units per volume or weight, or in any other manner that is relevant to the microorganism, e.g. colony forming units per gram (cfu/g).

This document is applicable to the blends of fertilizing products where a blend is a mix of at least two of the following component EU fertilising products categories: Fertilizers, Liming Materials, Soil Improvers, Growing Media, Plant Biostimulants, and where the following category Plant Biostimulants is the highest % in the blend by mass or volume, or in the case of liquid form by dry mass. If Plant Biostimulants is not the highest % in the blend, the European Standard for the highest % of the blend applies. In case a blend of fertilizing products is composed of components in equal quantity or in case the component EU fertilising products used for the blend have identical formulations[1], the user decides which standard to apply.

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 17708:2024, *Plant biostimulants — Preparation of sample for microbial analysis*

3 Terms and definitions

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

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 https://www.electropedia.org/

3.1
plant biostimulant
product stimulating plant nutrition processes independently of the product's nutrient content with the sole aim of improving the nutrient use efficiency, the tolerance to abiotic stress, the quality traits of the plant or the plant rhizosphere or the availability of confined nutrient in soil or rhizosphere

[SOURCE: EN 17724:2024, 3.1.1.8 [3], modified – editorial changes]

3.2
microorganism
any microbiological entity, including lower fungi, bacteria and viruses, cellular or non-cellular, capable of replication or of transferring genetic material, including dead or empty cells, microorganisms and non-harmful elements of the media on which they were produced

[SOURCE: EN 17724:2024, 3.2.2 [3], modified – "including dead or empty cells, microorganisms and non-harmful elements of the media on which they were produced" added]

[1] An example of such a blend is a product with 2 claimed functions consisting of a non-microbial plant biostimulant and an organic fertilizer composed of 1kg/kg of plant biostimulant from seaweed.

3.3
colony
localized visible accumulation of microbial mass (such as prokaryotes, bacteria, micromycetes, yeasts and fungi) or organisms (such as Dreissena species) developed on or in a solid nutrient medium from a viable particle or organism

Note 1 to entry: Frequently, microcolonies from nearby viable particles, before becoming visible, fuse into one macrocolony. The number of visible colonies is, therefore, usually an underestimate of the number of viable particles.

[SOURCE: ISO 6107:2021, 3.119 [4]]

3.4
product
portion of an identified plant biostimulant received in the laboratory for testing

3.5
laboratory sample
<in relation to chemical and physical testing>
final sample intended for laboratory testing and in relation to microbiological testing, each separate segment sample intended for laboratory testing

3.6
initial suspension
suspension (or solution) of the *laboratory sample* (3.5) in a defined volume of an appropriate diluent

3.7
Unit Potential Mycorrhizal
UPM
unit of counting for mycorrhiza

where

U is unit, spore or propagule of any type able to initiate mycorrhiza formation in a host plant's root;

P is potential, since the development of the symbiosis depend on different factors (soil, plant, agriculture practises, competition with other soil borne microorganisms, etc.);

M is mycorrhizal, since the inoculum is able to synthesize new mycorrhizae in association with plant roots depending on factors previously cited

EXAMPLE UPM per gram (% spores, % propagules) (*in vivo*, *in vitro*).

4 Principle of the method

The method for determining the concentration of microorganisms has been developed to provide a general method for the enumeration of the microorganisms present in the plant biostimulant. The results are expressed as the number of active units by volume or mass, or in any other way relevant to the microorganism, for example colony forming units per gram or the UPM for Mycorrhizae.

5 Sampling

Sampling is not part of the method specified in this document (see EN 17702-1:2024 [2]). If there is no specific International or European Standard, it is recommended that the parties concerned come to an agreement on this subject.

It is important that the laboratory receives a sample which is representative and has not been damaged or changed during transport or storage.

6 Preparation of sample for microbial analysis

The test sample shall be prepared from the laboratory sample in accordance with EN 17708:2024. If there is no specific International or European Standard, it is recommended that the parties concerned come to an agreement on this subject.

7 Method for the enumeration of microorganisms

7.1 General

When assessing the microbiological quality of plant biostimulants, it is often not enough to know only which microorganisms are present. In most cases, the quantitative aspect is equally important, which brings about the need to enumerate microorganisms [5]. This may be achieved in various ways: through direct examination (microscopy), by inoculating solid or liquid media. However, this document only covers enumeration using solid and liquid media.

Enumeration on solid media is based on the capacity of many microorganisms to produce colonies in or on agar media that can be recognized as such with the naked eye or with the aid of a simple magnifying glass.

If the level of bacteria is expected to be very low (less than 10 colonies in or on a plate at the lowest dilution), enumeration using liquid media is recommended (e.g. Most Probal Number (MPN)) to improve the statistical reliability of the results.

7.2 Enumeration using solid media

7.2.1 General

The different methods for enumeration using solid media (number of Petri dishes/dilutions, pour plate techniques, surface inoculation, incubation) are described in the different chapters below and/or in specific standards for microorganism detection/determination.

Petri dishes should be labelled with the sample number, dilution, date and any other desired information.

Dilutions should be selected to ensure that plates containing the appropriate number of colonies are obtained (see 7.2.6.1) and to overcome any possible inhibitory properties.

Use a separate sterile pipette for transfers from each dilution, except if working from the highest dilution to the lowest dilution.

7.2.2 Number of Petri dishes per dilution

For enumeration techniques in plant biostimulant(s), one plate per dilution shall be used with at least two successive dilutions. Two plates per dilution may also be used to improve reliability.

If only one dilution is used, then two plates of this dilution shall be used to improve reliability of the results.

For laboratories that do not operate under quality assurance principles, two plates per dilution shall be used to improve reliability of the results.

7.2.3 Pour plate techniques

Withdraw the defined volumes of the dilution to be examined, touching the tip of the pipette against the side of the tube to remove excess liquid adhering to the outside. Lift the sterile Petri dish lid just high enough to insert the pipette, then dispense the contents.

After removing the tempered agar medium from the water bath, blot the bottle dry with a clean towel to prevent water from contaminating the plates. Avoid spilling the medium on the outside of the container or on the inside of the plate lid when pouring. To avoid contamination of the medium, hold the bottle in a near horizontal position.

Also avoid setting down the bottle between pouring steps. Pour the agar medium at (44 ± 1) °C to (47 ± 1) °C into each Petri dish (generally 18 ml to 20 ml of agar in 90 mm Petri dishes and 45 ml to 50 ml in 140 mm Petri dishes, to obtain at least 3 mm of thickness) within 15 min of inoculation (to avoid aggregation of colonies). Avoid pouring the molten medium directly on the inoculum. Immediately mix the molten medium and the inoculum carefully so as to obtain a homogeneous distribution of the microorganisms within the medium, e.g. by gently moving the dish backwards and forwards, from side to side and in a circular direction. Allow to cool and solidify by placing the Petri dish on a cool horizontal surface (the solidification time of the agar shall not exceed 10 min).

7.2.4 Surface inoculation

7.2.4.1 General

Methods of plating designed to produce only surface colonies on agar plates have certain advantages. The morphology of surface colonies is easily observed, improving the analyst's ability to distinguish between different types of colonies.

Use pre-poured plates, of at least 3 mm of thickness of the agar medium, that are level and free from air bubbles and surface moisture.

To facilitate uniform spreading, the surface of solidified agar should be dried in accordance with EN ISO 11133:2014[2] [5] or as specified in the relevant International Standard so that the inoculum is absorbed within 15 min.

7.2.4.2 Spreading-spatula method

Using a sterile pipette, transfer the inoculum (usually 0,1 ml or 0,5 ml) of the liquid test sample, or of the initial suspension in the case of other samples, to the agar plate (90 mm or 140 mm in diameter, respectively). Repeat this step for the next decimal dilution (the colonies to be counted will then be present in a dilution step of 10^{-1} in the case of liquid sample materials and 10^{-2} in the case of other sample materials) and, if necessary, repeat for further decimal dilutions.

The limit of enumeration can be lowered by a factor of 10 by inoculating 1,0 ml of the test sample if liquid, or 1,0 ml of the initial suspension for other products, either on the surface of one large agar plate (140 mm) or on the surface of three small agar plates (90 mm). In both cases, if only one dilution is used, prepare duplicates by using two large plates or six small ones.

Using a spreading spatula made of glass, plastic or steel (for example made from a glass rod and shaped like a hockey stick of about 3,5 mm in diameter and 20 cm long, bent at right angles at about 3 cm from one end and flattened at the ends by heating), spread the inoculum as quickly as possible evenly over the agar surface without touching the side walls of the Petri dish. Allow the inoculum to absorb with the lids in place for about 15 min at room temperature.

In certain cases (as stated in the relevant International Standard), the inoculum may be deposited on a membrane then spread as described previously.

[2] As impacted by EN ISO 11133:2014/A1:2018 and EN ISO 11133:2014/A2:2020