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Rastlinski biostimulanti - Določanje kvasovk in plesni

Plant biostimulants - Determination of the yeast and mould content

Pflanzen-Biostimulanzien - Bestimmung des Gehalts an Hefen und Schimmelpilzen

Biostimulants des végétaux - Détermination de la teneur en levures et en moisissures

Ta slovenski standard je istoveten z: **EN 17707:2024**

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Plant biostimulants - Determination of the yeast and mould content

Biostimulants des végétaux - Détermination de la teneur en levures et en moisissures

Pflanzen-Biostimulanzien - Bestimmung des Gehalts an Hefen und Schimmelpilzen

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

CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

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EN 17707:2024 (E)

European foreword

This document (EN 17707:2024) 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of an identical text or by endorsement, at the latest by May 2025, and conflicting national standards shall be withdrawn at the latest by May 2025.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 17707:2022.

EN 17707:2024 includes the following significant technical changes with respect to CEN/TS 17707:2022:

- the Introduction has been updated and Table 1 has been removed;
- normative references have been updated;
- in Clause 3, new terms and definitions have been added and others have been revised;
- Annexes A, B and C have been revised;
- 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.

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EN 17707:2024 (E)**1 Scope**

This document specifies a horizontal method for the enumeration of yeasts and moulds present in plant biostimulants intended for use in agriculture, by means of the colony count technique after aerobic incubation at $25\text{ °C} \pm 2,5\text{ °C}$.

This document allows the enumeration of yeasts and moulds, in technical and formulated plant biostimulants, both in liquid and solid states. The method is applicable to microbial plant biostimulants except those composed of fungi or yeasts.

If necessary, yeasts and moulds enumerated can be identified using suitable identification tests.

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 formulations¹, 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 17702-1:2024, *Plant biostimulants — Sampling and sample preparation — Part 1: Sampling*

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

EN 17714:2024, *Plant biostimulants — Determination of microorganisms' concentration*

EN 17724:2024, *Plant biostimulants — Terminology*

3 Terms and definitions

For the purposes of this document, the terms and definition given in EN 17724:2024 and the following 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 yeast
mesophilic aerobic microorganism which, using mycological agar medium under the conditions described in this document, develops matt or shiny round colonies (3.3) on the surface of the medium, usually having a regular outline and a more or less convex surface

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.

3.2

mould

mesophilic aerobic filamentous microorganism which, on the surface of mycological agar medium under the conditions described in this document, usually develops flat or fluffy spreading colonies (3.3) often producing spores or conidia

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 [2]]

3.4

product

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

3.5

sample

portion of the product (3.4) that is used in the test to prepare the initial suspension

3.6

initial suspension

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

3.7

sample dilution

dilution of the initial suspension (3.6)

4 Principles

4.1 General

This method shall be followed for the enumeration of colonies on a selective agar medium.

4.2 Plate count method

The plate count consists in the following steps:

- preparation of poured plates, or spread plates using a specific culture medium. Depending on the expected number of colonies, a specified quantity of the sample (if the product is liquid), or of an initial suspension (in the case of other products), or decimal dilutions of the sample/suspension shall be inoculated;
- aerobic incubation of the plates shall be $25\text{ °C} \pm 2,5\text{ °C}$ for 3 days to 5 days;
- calculation of the number of colony-forming units (CFU) of yeasts and moulds per gram or per millilitre of sample from the number of colonies obtained on plates chosen at dilution levels producing countable colonies. Moulds and yeasts may be counted separately, if necessary.

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NOTE An alternative condition for incubation may be $22,5\text{ °C} \pm 2,5\text{ °C}$, for 5 days to 7 days, using the culture medium without antibiotic. If necessary, to distinguish yeast colonies from bacterial colonies, the identity of any doubtful colonies is confirmed by examination with a binocular magnifier or microscope.

5 Diluent and culture media

5.1 General

The following diluents and culture media should be used since they are suitable for the enumeration of yeasts and moulds. Other diluents and culture media may be used if they have been demonstrated to be suitable for use.

Diluent and culture media should be prepared using the descriptions provided in this document or from dehydrated culture media, according to the instructions from the manufacturer.

NOTE Ready-to-use media may be used when their composition and/or growth yields are comparable to those of the formulae given in the present document.

5.2 Diluent

In the preparation of the initial suspension and further decimal dilutions, the Phosphate Buffer Solution (PBS) should be used, and the recipe described in A.2 shall be followed. Another suitable diluent (e.g. Buffered Peptone Water) may be used. The preparation of this alternative diluent is not part of the method specified in this document: EN 17708:2024 shall be used.

5.3 Culture media

See Annex A for the list and recipes of the possible media to be used in the inoculation by plating technique of the initial suspension and the further decimal dilutions.

6 Apparatus and glassware

Use the laboratory equipment, apparatus and glassware typical of microbiological laboratory. See EN 17708:2024 for a detailed list.

7 Handling of plant biostimulants and sampling

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

Sampling is not part of the method specified in this document: EN 17702-1:2024 shall be used.

If necessary, the product to be tested may be equilibrated at room temperature before starting the analysis.

8 Procedure

8.1 General

Use sterile material, equipment and aseptic techniques to prepare the sample, initial suspension and dilutions. In the case of the preparation of the initial suspension, the time that elapses between the end of the preparation and the moment the inoculum comes into contact with the culture medium shall not exceed 45 min, unless specifically mentioned in the established protocols or documents.

Appropriate negative controls (diluent-only) should be run concurrently with the sample serial dilutions. This step can be performed by incubating an aliquot of the diluent (i.e. 9 ml) at the same conditions of the test to verify the absence of turbidity to assess the sterility of the diluent. Or, alternatively, 1 ml of the