SLOVENSKI STANDARD
SIST-TS CEN/TS 17719:2023

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Rastlinski biostimulanti - Določanje števila na anaerobnih mikrotitrskih ploščah

Plant biostimulants - Determination of the anaerobic plate count

Pflanzen-Biostimulanzien - Bestimmung der anaeroben Keimzahl

Biostimulants des végétaux - Détermination du dénombrement sur plaque des germes anaérobies

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65.080 Gnojila Fertilizers

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TECHNICAL SPECIFICATION

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ICS 65.080

English Version

Plant biostimulants - Determination of the anaerobic plate count

Biostimulanzien für die pflanzliche Anwendung - Bestimmung der anaeroben Keimzahl

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

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European foreword

This document (CEN/TS 17719:2022) has been prepared by Technical Committee CEN/TC 455 “Plant Biostimulants”, the secretariat of which is held by AFNOR.

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This document has been prepared under a Standardization Request given to CEN by the European Commission and the European Free Trade Association.

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.

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Introduction

This document was prepared by the experts of CEN/TC 455 “Plant Biostimulants”. 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 the European Parliament and of the Council of 5 June 2019 laying down rules on the making available on the market of EU fertilising products (“FPR” or “Fertilising Products Regulation”). This standardization request, presented as M/564, also contributes to the Communication on “Innovating for Sustainable Growth: A Bio economy for Europe”. Working Group 5 “Labelling and denominations” was created to develop a work program as part of this standardization request.

Technical Committee CEN/TC 455 “Plant Biostimulants” was established to carry out the work program that will prepare a series of standards. The interest in 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.

Biostimulants used in agriculture can be applied in multiple ways: on soil, on plants, as seed treatment, etc. A microbial plant biostimulant consists of a microorganism or a consortium of microorganisms, as referred to in Component Material Category 7 of Annex II of the EU Fertilising Products Regulation 2019/1009 [1].

Table 1 summarizes many of the agro-ecological principles and the role played by biostimulants.

Table 1 — Agro-ecological principles and the role played by biostimulants

Increase biodiversity
By improving soil microorganism quality/quantity
Reinforce biological regulation and interactions
By reinforcing plant-microorganism interactions
— symbiotic exchanges i.e. *Mycorrhizae*
— symbiotic exchanges i.e. *Rhizobiaceae/Fava*
— secretions mimicking plant hormones (i.e. *Trichoderma*)
By regulating plant physiological processes
— e.g. growth, metabolism, plant development…
Improve biogeochemical cycles
— improve absorption of nutritional elements
— improve bioavailability of nutritional elements in the soil
— stimulate degradation of organic matter

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 provides a horizontal method for enumeration of microorganisms that are able to grow and form colonies in a solid medium after anaerobic incubation at 30 °C.

The method is applicable to microbial plant biostimulants for verifying that the concentration of anaerobes does not exceed the respective limits outlined in the EU Regulation on Fertilising Products [1].

This method does not apply to the microbiological monitoring of the environment in which microbial plant biostimulants are manufactured.

No information about potential human pathogens can be inferred from anaerobic plate counts.

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.

CEN/TS 17708, *Plant biostimulants - Preparation of sample for microbial analysis*

CEN/TS 17724, *Plant biostimulants - Terminology*

EN ISO 7218:2007,[1] *Microbiology of food and animal feeding stuffs - General requirements and guidance for microbiological examinations (ISO 7218:2007)*

EN ISO 11133:2014,[2] *Microbiology of food, animal feed and water - Preparation, production, storage and performance testing of culture media (ISO 11133:2014)*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in CEN/TS 17724 and the following apply.

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

— IEC Electropedia: available at https://www.electropedia.org/

— ISO Online browsing platform: available at https://www.iso.org/obp

3.1
microorganism
any microbiological entity, including lower fungi, bacteria and viruses, cellular or non-cellular, capable of replication or of transferring genetic material

[SOURCE: Regulation (EC) No 1107/2009, Article 3, point 15]

[1] As impacted by EN ISO 7218:2007/A1:2013.

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

3.2
facultative organism
microorganism capable of both aerobic and anaerobic metabolism

[SOURCE: EN ISO 11139:2018, 3.114]

3.3
obligate anaerobe
organism that only lives and grows in the absence of molecular oxygen

[SOURCE: EN ISO 11139:2018, 3.186]

3.4
viable count
value established from enumeration of recoverable colony-forming units

[SOURCE: EN ISO 11139:2018, 3.386]

4 Principle

4.1 General

Viable anaerobic bacteria are enumerated by the plate count technique under an anaerobic atmosphere [2]. Caution shall be exercised when applying the method since isolates may be pathogenic.

4.2 Brief description

A fixed amount of a dilution of the test sample is placed in an empty Petri dish and mixed in a specified melted agar culture medium to create a pour plate.

The plates are incubated at 30 °C ± 1 °C under anaerobic conditions for 48 h to 72 h.

The number of microorganisms per gram or millilitre of test sample is calculated as specified in Clause 9.

5 Culture media and reagents

For current laboratory practices, CEN/TS 17708 and EN ISO 11133:2014[2] shall be used.

Composition of Anaerobic Agar (ANA) [3] and reagents and their preparation are described in Annex B.

6 Equipment and consumables

6.1 Special apparatus, anaerobic incubator, BBL GasPak™[3] or equivalent, equipped with GasPak™ hydrogen and CO_2 generator envelopes with an anaerobic indicator.

6.2 Apparatus for dry sterilization (oven) or wet sterilization (autoclave), according to EN ISO 7218:2007[1] shall be used.

6.3 Drying cabinet or incubator, capable of operating at 30 °C to 35 °C ± 1 °C.

[3] BBL GasPak™ and Stomacher® are examples of suitable products available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN of these products.

6.4 Water bath, capable of operating at 47 °C ± 2°C.

6.5 pH-meter, having an accuracy of calibration of ± 0,1 pH unit at 25 °C.

6.6 Sterile graduated pipettes or automatic pipettes, of nominal capacities of 0,1 ml, 0,5 ml, 1 ml, and 10 ml.

6.7 Sterile Petri dishes, with a diameter of approximately 90 mm and (optional) large size (diameter approximately 140 mm).

6.8 Refrigerator, capable of operating at 5 °C ± 3 °C.

6.9 Peristaltic blender (Stomacher®[3]) with 400 ml sterile bags.

6.10 Blender motor and jars or vortex.

7 Preparation of test sample

7.1 General

To ensure a truly representative analytical unit, agitate liquids or free flowing materials until the contents are homogeneous. If the sample unit is a solid, obtain the analytical unit by taking a portion from several locations within the sample unit. To reduce the workload, the analytical units may be combined for analysis. It is recommended that a composite contains no more than five analytical units.

General rules for the preparation of the initial suspension for microbiological examination are described in CEN/TS 17708.

A representative sample of the product should be prepared taking into consideration the different formulations of the plant biostimulants.

7.2 Liquid – water-based formulations

Aseptically add 25 ml of the product (the analytical unit) in a 400 ml sterile Stomacher® bag (6.9) or in a blender jar (6.10) containing 225 ml of an appropriate sterile diluent (i.e. buffered peptone water) maintained at room temperature. Blend, stomach or vortex as required for thorough mixing.

7.3 Liquid – oil-based (emulsifiable concentrate - EC) formulations

Aseptically add 25 ml of the product (the analytical unit) in a 400 ml sterile Stomacher® bag (6.9) or in a blender jar (6.10) containing 225 ml of an appropriate sterile diluent (i.e. buffered peptone water) maintained at room temperature. Blend, stomach or vortex as required for thorough mixing.

7.4 Solid – wettable powder (WP) formulations

Aseptically add 25 g of the product (the analytical unit) in a 400 ml sterile Stomacher® bag (6.9) containing 225 ml of an appropriate sterile diluent (i.e. buffered peptone water) maintained at room temperature. Homogenize the mixture 2 min at higher speed with a Stomacher® (6.9).

7.5 Solid – water dispersible granules (WDG) formulations

Aseptically add 25 g of the product (the analytical unit) in a 400 ml sterile Stomacher® bag (6.9) containing 225 ml of an appropriate sterile diluent (i.e. buffered peptone water) maintained at room temperature. Homogenize the mixture 2 min at higher speed with a Stomacher® (6.9).