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BSI Standards Publication

Plant biostimulants — Determination of *Azotobacter* spp.

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National foreword

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European foreword

This document (CEN/TS 17709:2022) has been prepared by Technical Committee CEN/TC 455 “Plant biostimulants”, the secretariat of which is held by AFNOR.

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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 5 June 2019 laying down rules on the making available on the market of EU fertilizing 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”. The Working Group 5 “Labelling and denominations”, was created to develop a work program as part of this request. The 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 plant 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.

This document is applicable to all biostimulants in agriculture based on live microorganisms belonging to the genera *Azotobacter*.

The Table 1 below 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/Faba*
- secretions mimicking plant hormones (i.e. *Trichoderma*)
By regulating plant physiological processes
- for ex 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 was developed to provide the methodology for the enumeration and determination of *Azotobacter* sp. in plant biostimulant products in accordance with the Regulation (EU) 2019/1009 of the European Parliament and of the Council [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.

CEN/TS 17702-1, *Plant biostimulants — Sampling and sample preparation — Part 1: Sampling*

CEN/TS 17724, *Plant biostimulants — Terminology*

3 Terms and definitions

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

4 Enumeration of *Azotobacter* spp.

4.1 General

This procedure is meant to determine the number of colony-forming units (CFU) of the above mentioned bacteria, per gram, per millilitre, per square centimetre or per sampling device. The method, in order to be fast, cheap, repeatable, is based on serial dilutions and plating.

4.2 Sample preparation

4.2.1 General

A representative sample of the products to be analysed according to CEN/TS 17702-1 shall be prepared according to following procedure which takes into consideration the different formulations of biostimulants based products.

4.2.2 Liquid (based water) formulations

Dispense 25 ml of sample (or more for low concentrated products) in 225 ml of sterile Phosphate Buffer Solution (PBS) maintained at room temperature, in a flask and shake for 10 min or more until the distribution is optimal, with a magnetic stirrer at half speed [6].

4.2.3 Liquid - based oil, emulsifiable concentrate (EC) formulations

Dispense 25 ml of sample (or more for low concentrated products) in 225 ml of sterile Phosphate Buffer Solution (PBS) maintained at room temperature, in a flask and shake for 10 min or more until the distribution is optimal, with a magnetic stirrer at half speed [6].

4.2.4 Solid - Wettable Powder (WP) formulations

Dispense 25 g of sample (or more for low concentrated products) in 225 ml of sterile Phosphate Buffer Solution (PBS) maintained at room temperature, in a flask and shake for 20 min or more until the distribution is optimal, with a magnetic stirrer at half speed [6].