TECHNICAL SPECIFICATION

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Soil quality — Measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates

Qualité du sol — Mesure en microplaques de l'activité enzymatique dans des échantillons de sol en utilisant des substrats fluorogènes

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Contents

Page

Foreword

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The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

In other circumstances, particularly when there is an urgent market requirement for such documents, a technical committee may decide to publish other types of document:

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

ISO/TS 22939 was prepared by Technical Committee ISO/TC 190, *Soil quality*, Subcommittee SC 4, *Biological methods*.

Introduction

Micro-organisms are responsible for many key processes in the cycle of elements. Enzymes are responsible for the degradation of organic molecules and their mineralization. The main postulate is the microbial origin of soil enzymes, even if plant root exudates include enzymes. Extracellular enzymes in soil play key roles in the biodegradation of organic macromolecules. The simultaneous monitoring of several enzyme activities important in the biodegradation of organic compounds and mineralization of C, N, P and S in soil may reveal harmful effects caused by chemicals and other anthropogenic impacts. However, the measurements carried out under selected laboratory conditions using artificial substrates cannot be a substitute for the actual rate of enzymatic processes in soil *in situ*.

Soil quality — Measurement of enzyme activity patterns in soil samples using fluorogenic substrates in micro-well plates

1 Scope

This Technical Specification specifies a method for the measurement of several enzyme activities simultaneously in soil samples. Enzyme activities of soil vary seasonally and depend on the chemical, physical and biological characteristics of soil. Its application for the detection of harmful effects of toxic chemicals or other anthropogenic impacts depends on the simultaneous comparison of enzyme activities in a control soil similar to the test soil, or on exposure tests with chemicals or treatments.

2 Normative references

The following referenced documents are indispensable for the application 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.

ISO 10381-6, *Soil quality — Sampling — Part 6: Guidance on the collection, handling and storage of soil under aerobic conditions for the assessment of microbiological processes, biomass and diversity in the laboratory*

ISO 10390, *Soil quality — Determination of pH*

ISO 10694, *Soil quality — Determination of organic carbon and total carbon after dry combustion (elementary analysis)*

3 Abbreviated terms

E.C. Enzyme code number defined by the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (NC-IUBMB)

SOM Soil organic matter content

MUB Modified universal buffer

4 Principle

This Technical Specification describes a method for the simultaneous measurements of several enzymes in soil samples. It is based on the use of soil samples diluted in buffer containing fluorogenic substrates, which are incubated for 3 h at (30 ± 2) °C in multi-well plates. After the incubation the enzyme activities are measured as fluorescence with a plate-reading fluorometer (References [1] and [2] in the Bibliography). The method described is based on dried standard and substrate plates enabling storage and limiting bias due to differences between reagent batches, and also enabling comparison between reagent batches. Annex A describes a method utilizing freshly prepared reagents, which has a clearly defined and exact incubation period. The advantage of the use of freshly prepared substrates is that an instrument for lyophilization is not required.