SLOVENSKI STANDARD

SIST-TP CEN/TR 15214-3:2006

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Karakterizacija blata ï Ugotavljanje prisotnosti in ġtevila Echerichia coli v blatu, zemljini, izboljġevalcih tal, rastnih substratih in bio-odpadkih - 3. del: Makrometoda (najverjetnejġe ġtevilo ï MPN) v tekoļ em mediju

Characterization of sludges - Detection and enumeration of Escherichia coli in sludges, soils, soil improvers, growing media and biowastes - Part 3: Macromethod (Most Probable Number) in liquid medium

Charakterisierung von Schlämmen - Quantitativer Nachweis von Escherichia coli in Schlämmen, Böden, Bodenverbesserungsmitteln, Kultursubstraten sowie Bioabfällen - Teil 3: Makroverfahren in Flüssigmedium (MPN-Verfahren)

Caractérisation des boues - Détection et dénombrement des Escherichia coli dans les boues, les sols, les amendements du sol, les supports de culture et les biodéchets - Partie 3 : Macrométhode (nombre le plus probable) par ensemencement en milieu liquide

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This Technical Report was approved by CEN on 3 September 2005. It has been drawn up by the Technical Committee CEN/TC 308.

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Foreword

This Technical Report (CEN/TR 15214-3:2006) has been prepared by Technical Committee CEN/TC 308 “Characterization of sludges”, the secretariat of which is held by AFNOR.

This Technical Report does not replace any existing CEN method.

The standard is divided into three parts:

Part 1 gives a membrane filtration for quantification

Part 2 gives a miniaturised semi-quantitative MPN method and

Part 3 gives a semi-quantitative macro method

Introduction

Sludges, soils, soil improvers, growing media and biowastes can contain pathogenic micro-organisms such as *Salmonella* spp. which occur mainly in the intestinal tract of humans and animals and are transmitted through faecal contamination. The use of such contaminated materials in agriculture may cause outbreaks of infection due to the production of contaminated food and animal feedstocks. It may also be transmitted to wild animals. There is a need to monitor the efficacy of the storage and treatment processes to control pathogens such as *Salmonella* spp., and application rates to land.

Escherichia coli is a non-pathogenic, Gram negative bacterium with faecal origin. Consequently, it can be used as an indicator of faecal contamination. It can also be used to monitor the effectiveness of pasteurization or disinfection treatments but it is comparatively sensitive (to heat, high pH) and cannot therefore reflect the behaviour of all pathogens in these materials. Suitable quality control procedures, at least those described in ISO 8199, have to be applied.

WARNING — "Waste and sludge samples can contain hazardous and inflammable substances. They can contain pathogens and be liable to biological action. Consequently, it is recommended that these samples should be handled with special care. The gases which can be produced by microbiological activity are potentially inflammable and will pressurise sealed bottles. Exploding bottles are likely to result in infectious shrapnel and/or pathogenic aerosols. Glass bottles should be avoided wherever possible. National regulations should be followed with respect to microbiological hazards associated with this method".

1 Scope

This part of the CEN Technical Report specifies a most probable number (MPN) method for the quantitative detection of *Escherichia coli* in sludges, soils, soil improvers, growing media and biowastes. It allows further differentiation within the test than part 2 of this standard. It is suitable to evaluate the log reduction of *E.coli* through treatment as well as the quality of the end product.

The method is for material with dry residues of more than 10 %.

For materials with dry residues less than 10 %, the procedure given in CEN/TR 15214-1 should be used.

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.

EN 12880:2000, *Characterisation of Sludges - Determination of Dry Residue and Water Content*.

ISO 8199, *Water quality – General guide to the enumeration of micro-organisms by culture*.

3 Terms and definitions

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

3.1
Escherichia coli
Escherichia coli, which belongs to the family of Enterobacteriaceae, are Gram-negative, non-sporulating, rod-shaped, lactose positive bacteria able to grow at 44 °C. Most *E.coli* strains are able to produce indole from tryptophane and are β-glucuronidase-positive

3.2
method definition
for the purpose of the present method, the following *E. coli* definition shall apply: - β-glucuronidase-positive and able to hydrolyse 4-methylumbelliferyl-B-D-glucoronide (MUG) when at an incubation temperature of 44 °C in the specified liquid medium. In addition, indole shall be produced from tryptophan and gas produced from lactose

3.3
dry residue
the dry mass portion of the sample obtained after the specified drying process. It is expressed as percent or in grams per kilogram

[EN 12880:2000, 3.1].

4 Principle

This method is based on that described to Schindler 1991 (Fluorcult™ or equivalent).

a) Suspension of the sample in 0.9 % m/V sodium chloride;

b) Serial dilutions of this suspension in the same diluent;

c) Transfer 1ml of these diluted suspensions into 3 tubes containing 9 ml MUG fluorocult lauryl sulfate broth;

d) Incubation at (44 ± 1) °C for (40 ± 4) h;

e) Detection of gas production, indole formation and fluorescence;

f) Quantification by the MPN technique.

5 Apparatus

With the exception of equipment supplied sterile, the glassware shall be sterilised in accordance with the instructions given in ISO 8199.

Usual microbiological laboratory equipment, and in particular:

5.1 Wide-mouth glass flasks or beakers for example 125 ml, 200 ml, 500 ml and 2 000 ml.

5.2 Thermostatic incubators regulated at (36 ± 2) °C and (44 ± 1) °C (static).

5.3 Autoclave (Steam sterilizer).

5.4 Refrigerator.

5.5 Sterile plastics culture dishes, with lid of about 90 mm in diameter.

5.6 Graduated pipettes, of nominal capacities 1 and 10 ml.

5.7 Apparatus for shaking the culture tubes.

5.8 Culture tubes, 25 ml capacity, or equivalent containers.

5.9 Vortex mixer suitable for of 25 ml capacity culture tubes or equivalent containers.

5.10 Durham – tubes.

5.11 pH meter, with temperature compensation and pH measuring cell.

5.12 Boiling water bath.

5.13 UV-lamp (366 nm).

6 Sampling and hazards

6.1 Introduction

Take samples of at least 100 g wet weight and deliver them to the laboratory as quickly as possible, preferably chilled at (5 ± 3) °C.

6.2 General

Samples are liable to ferment, particularly if untreated, and may contain pathogenic micro-organisms. It is essential to keep them away from any food or drink, and to protect any cuts. Bursting glass bottles containing sludge can produce micro-organism contaminated shrapnel. Plastic bottles can also burst and produce a hazardous spray and aerosol.

See also the Warning note in Introduction.

6.3 Storage

It is not advisable to store samples in the open laboratory. If samples are to be stored, store them at (5 ± 3) °C.

6.4 Handling

Cleanliness when working is essential. When handling sludge samples, it is necessary to wear gloves, face and eye protection, and sufficient body protection to guard against bottles bursting. The gas evolved is usually flammable, so all equipment used in the vicinity shall be flame proof to avoid any source of ignition.

See also the Warning note in the Introduction.

7 Reagents, diluents and culture media

7.1 General instructions

To ensure reproducible results, prepare culture media and diluents using either constituents of uniform quality and chemicals of recognised analytical grade, or a dehydrated diluent or complete medium prepared following the manufacturer's instructions. Prepare them with demineralised or distilled water free from substances capable of inhibiting growth under the test conditions. If the media are not used immediately, preserve them in the dark at (5 ± 3) °C for up to one month in conditions avoiding any alterations in their composition.

NOTE The use of chemicals of other grades is permissible providing that they are shown to be of equivalent performance in the test.

7.2 MUG(4-methyl-umbelliferyl-β-D-glucuronide) Lauryl sulphate broth (e.g. Fluorocult™ or equivalent) prepared according the manufacturer or :

Mix the following substances in 1 000 ml water in a 2 000 ml flat bottom flask, while heating in a boiling water bath (5.12):

— 20,0 g trypsin digested peptone from meat

— 5,0 g lactose

— 5,0 g sodium chloride

— 0,1 g sodium lauryl sulfate

— 2,75 di-potassium hydrogen phosphate

— 2,75 g potassium di-hydrogen phosphate

— 1,0 g L- tryptophan

— 0,1 g 4-methyl-umbelliferyl- ß-D-glucuronide