SLOVENSKI STANDARD
SIST-TP CEN/TR 15214-1:2006

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Karakterizacija blata – Ugotavljanje prisotnosti in števila Echerichia coli v blatu, zemljini, izboljševalcih tal, rastnih substratih in bio-odpadkih - 1. del: Metoda membranske filtracije za kvantifikacijo

Characterization of sludges - Detection and enumeration of Escherichia coli in sludges, soils, soil improvers, growing media and biowastes - Part 1: Membrane filtration method for quantification

Charakterisierung von Schlämmen - Quantitativer Nachweis von Escherichia coli in Schlämmen, Böden, Bodenverbesserungsmitteln, Kultursubstraten sowie Bioabfällen - Teil 1: Membranfiltrationsverfahren zur quantitativen Bestimmung

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 1 : Méthode de quantification par filtration sur membrane

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

Management Centre: rue de Stassart, 36 B-1050 Brussels

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Contents

Page

Foreword

This Technical Report (CEN/TR 15214-1:2006) 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 macromethod.

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 foodstocks. They 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 a 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 membrane filtration procedure for the quantitative detection, by culture of individual colonies on chromogenic agar media, of *Escherichia coli*. in sludges, soils, soil improvers, growing media and biowastes. This part of the Technical Report is not suitable for materials whose treatment will significantly reduce bacterial levels to less than 10 viable *E. coli* per g wet weight, such as lime addition, drying or pasteurisation. A liquid enrichment and most probable number estimation method may be suited for such purpose.

This membrane filtration method is not appropriate for enumeration and detection of other coliform bacteria without modifications to the chromogenic agar media.

It is suitable to evaluate the log reduction of *E.coli* through treatment, as well as the quality of the end product.

This method is for materials with dry residues less than 20 %. For materials with dry residues greater than 20 % and low numbers of *E. coli*, CEN/TR 15214-2 and CEN/TR 15214-3 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 guidance on 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, belongs to the family of *Enterobacteriaceae*, is Gram-negative, non-sporulating, rod-shaped, lactose positive and able to grow at 44 °C. Most *E. coli* strains are able to produce indole from tryptophan and are β-glucuronidase-positive

3.2
method definition
β-glucuronidase-positive and able to hydrolyse 5-bromo-4-chloro-3-indolyl-β-glucuronide (BCIG) when growing on an MLG agar medium at the temperature of 44 °C

3.3
cfu, colony forming unit
growth of individual bacterial cells into visible colonies on agar media, including on membrane filters overlaying the agar media

3.4
vegetative bacteria
those bacteria which are capable of normal growth in broth or on agar media without pre-culture resuscitation

3.5
sub-lethally damaged bacteria
those bacteria which have been stressed but not killed by storage or subsequent treatment by, for example, mesophilic anaerobic digestion, lime stabilisation or composting

3.6
resuscitation
stimulation to vegetative growth of sub-lethally damaged bacteria previously incapable of growth on agar media

3.7
quantitative resuscitation
stimulation to vegetative growth of sub-lethally damaged bacteria recovered discretely on a membrane filter, prior to transfer to chromogenic medium for growth of individual colonies

3.8
dry residue
the dry mass portion of the material obtained after the specified drying process. It is expressed as percent or in grams per kilogram [EN 12880:2000, 3.1]

4 Principle

The diluted sludge sample is filtered, the membrane filter recovered aseptically and incubated on membrane lactose glucuronide agar (MLGA), initially at (30 ± 1) °C for (4 ± 0,5) h. Subsequently, the temperature is increased to (44 ± 1) °C for (14 ± 2) hours. The presence of *E. coli* is indicated by green colonies resulting from the hydrolysis of BCIG. The method has a limit of detection of approximately 10 cfu g^{-1} wet weight sludge, dependent on the solids content which at high concentrations (> 20 % (w/v)) may restrict filtration of the sample volume through the membrane if not first diluted.

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 Apparatus for sterilisation by dry heat (oven) or steam (autoclave).

5.2 Thermostatic incubator(s) regulated at (30 ± 1) °C and/or (44 ± 1) °C.

5.3 Homogeniser (e.g. Stomacher®, Seward Laboratories or equivalent).

5.4 Membrane filters (0,45 µm gridded, cellulose nitrate, 47 mm diameter or equivalent).

5.5 Glass fibre filter discs (47 mm diameter, e.g. Sartorius 13430-0475 or equivalent nominal pore size).

5.6 Vacuum pump.

5.7 Vacuum manifold.

5.8 Sterile homogeniser bags, 250 ml volume, with or without integrated mesh to exclude large particulate matter (e.g. Stomacher®, Seward Laboratories 6041, 6041/STR or equivalent).

5.9 Sterile Petri dishes, 50 mm in diameter, for holding MLGA medium.

5.10 Sterile test tubes of 20 ml volume, or flasks with similar capacity.

5.11 Automatic pipettes, capable of dispensing 1,0-5,0 ml volumes.

5.12 Sterile pipettes, glass or disposable plastic ware, capable of dispensing 1 ml and 10 ml volumes.

5.13 Sterile tips for automatic pipettes.

5.14 Sterile conical centrifuge tubes, 50 ml volume, disposable plastic.

5.15 Tweezers, capable of sterilisation by immersion in ethanol and subsequent flaming.

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 the 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 Maximal recovery diluent (MRD)

— Bacteriological peptone (Oxoid L37 or equivalent)	1 g
— Sodium chloride	8,5 g
— Demineralised or distilled water	to 1000 ml