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English Version

**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**

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

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

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-glucuronide (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 (Fluorocult™ or equivalent).

- a) Suspension of the sample in 0.9 % m/V sodium chloride;
- b) Serial dilutions of this suspension in the same diluent;