Australian Standard®

Water microbiology

Method 23: Soils, sediments, sludges, slurries and bio-solids—Procedures for sample preparation

AS 4276.23:2016

PREFACE

This Standard was prepared by the Standards Australia Committee FT-020, Water Microbiology.

The objective of this Standard is to provide procedures for preparation of high-particulate-liquid (slurries), solid and semi-solid environmental matrices for microbiological examination. These matrices are frequently analysed for a range of microorganisms, particularly faecal indicators and pathogens. In particular, biosolids are tested to determine fitness-for-purpose for various end uses. There is a lack of peer reviewed procedures for the preparation of these matrices for microbiological examination and a demand exists by laboratories seeking technical accreditation for analysing them.

The main use of this Standard will be for the testing of biosolids for various end uses as part of the process of ensuring that public health is not compromised through the use of biosolids with inappropriate pathogen levels. The procedures are also applicable for preparation of soils, sediments and sludges for microbiological analysis. Examples of matrices of interest are those that relate to soil irrigation using reclaimed and agricultural wastewater, bacterial partitioning into sediments in aquatic environments, and waste treatment efficacy with respect to reduction of microbial/pathogen loads in sludges.

The terms 'normative' and 'informative' have been used in this Standard to define the application of the appendix to which they apply. A 'normative' appendix is an integral part of a Standard, whereas an 'informative' appendix is only for information and guidance.

FOREWORD

Biosolids are regarded as a valuable resource. The National Water Quality Management Strategy publication, *Guidelines for Sewerage Systems—Biosolids Management*, states that there is a 'need to ensure that public health is not compromised through using biosolids with inappropriate pathogen levels'. This new Standard would facilitate more consistent and effective testing of biosolids nationally for pathogenic and indicator microorganisms.

Microorganisms in solid and semi-solid matrices are often adsorbed onto, or absorbed within, particulate material. This may produce uneven physical distributions, resulting in decreased subsample representativeness, and decreased overall test precision and accuracy. The goal of this Standard is effective sample homogenization, allowing subsequent accurate and reproducible quantitative dispensing and/or dilution. Results in subsequent analyses may be semi-quantitative or quantitative.

Most Probable Number (MPN), presence-absence (PA) and direct-agar plate-based techniques require homogenous sample suspensions for representative analysis. MPN and PA techniques allow for the presence of substantial amounts of matrix material in the growth medium. Therefore, physical separation of target organism from matrix is not necessary.

Direct-agar plating techniques such as spread-plate or membrane filtration are subject to matrix interference such as membrane filter and/or pipette clogging. The inability to distinguish target colonies from particulate matrix material is another potential interference. Mitigation of such interference may be achieved through physical separation of microorganisms from the matrix and/or dilution of sample such that matrix no longer impedes or confounds the analysis.

Analysis of samples containing large-size interfering particles (>1–2 mm diameter) may require continuous resuspension during liquid handling to prevent differential particle settling; and dilution or separation of target microorganisms from particles where such particles interfere with culture based enumeration.

Separation of target microorganisms from matrix particles is promoted through the use of physical disruption in the presence of a homogenization solution containing a surfactant and dispersing agent.

METHOD

1 SCOPE

This Standard sets out a method for the preparation of soils, sediments, sludges [including high-particulate-content liquids (slurries)] and biosolids for culture-based bacterial analysis utilizing selective growth media and/or conditions, including most probable number (MPN), presence/absence (PA) and agar plating techniques. Examples of analytes include (but are not limited to): *E. coli*, thermotolerant coliforms, enterococci, *Salmonella* spp., *Vibrio cholerae*, *Clostridium perfringens*, *Campylobacter jejuni/coli*, *Burkholderia pseudomallei*, iron and sulphur bacteria and hydrocarbon-degrading bacteria.

NOTE: Flow diagram of the procedure for sample preparation for soils, sediments, sludges [including high-particulate-content liquids (slurries)] is given in Appendix A.

Although sample preparation for protozoans, helminths and viruses in these matrices is not within the scope of this Standard, some guidance on sample preparation is given in Appendix B.

2 REFERENCED DOCUMENTS

The following documents are referred to in this Standard:

AS

2031 Water quality—Sampling for microbiological analysis (ISO 19458:2006, MOD)

AS/NZS

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4276.1 Method 1: General information and procedures (ISO 8199:2005, MOD)