SLOVENSKI STANDARD
SIST EN 16421:2015

01-februar-2015

Vpliv materialov na pitno vodo - Spodbujanje mikrobiološke rasti

Influence of materials on water for human consumption - Enhancement of microbial growth (EMG)

Einfluss von Materialien auf Wasser für den menschlichen Gebrauch - Förderung des mikrobiellen Wachstums

Influence des matériaux sur l'eau destinée à la consommation humaine - Stimulation de la croissance microbienne (SCM)

Ta slovenski standard je istoveten z: **EN 16421:2014**

ICS:

07.100.20	Mikrobiologija vode	Microbiology of water
13.060.20	Pitna voda	Drinking water
67.250	Materiali in predmeti v stiku z živili	Materials and articles in contact with foodstuffs

SIST EN 16421:2015 **en,fr**

EUROPEAN STANDARD

NORME EUROPÉENNE

EUROPÄISCHE NORM

EN 16421

December 2014

ICS 07.100.20

English Version

Influence of materials on water for human consumption - Enhancement of microbial growth (EMG)

Influence des matériaux sur l'eau destinée à la consommation humaine - Stimulation de la croissance microbienne (SCM)

Einfluss von Materialien auf Wasser für den menschlichen Gebrauch - Förderung des mikrobiellen Wachstums

This European Standard was approved by CEN on 25 October 2014.

CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration. Up-to-date lists and bibliographical references concerning such national standards may be obtained on application to the CEN-CENELEC Management Centre or to any CEN member.

This European Standard exists in three official versions (English, French, German). A version in any other language made by translation under the responsibility of a CEN member into its own language and notified to the CEN-CENELEC Management Centre has the same status as the official versions.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

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

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Contents

Page

Foreword

This document (EN 16421:2014) has been prepared by Technical Committee CEN/TC 164 “Water supply”, the secretariat of which is held by AFNOR.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by June 2015, and conflicting national standards shall be withdrawn at the latest by June 2015.

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

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

Water intended for human consumption comes into contact with construction products during storage, transportation and distribution, including water systems inside buildings. The materials used in these products are selected on the basis of technical requirements and criteria regarding their influence on the water quality, e.g. release of substances and effects on odour, flavour or colour of the water. However, water quality problems may also arise when such materials enhance the multiplication of micro-organisms.

A test method to determine the enhancement of microbial growth is required as organic substances present in non-metallic materials (either as ingredients, contaminants or process by-products) are capable of being utilized by micro-organisms and can give rise to a noticeable deterioration in the organoleptic, physical or microbiological quality of the water with which they are in contact. Microbial growth may occur in the water itself or at the material/water interface.

Materials with the potential of supporting microbial growth do not necessarily lead to a deterioration in water quality in every situation due to the influence of various environmental factors, e.g. microbial quality of the water, temperature, presence of residual disinfectant or other growth limiting factors.

The purpose of this standard is to describe three European test methods that can be applied to determine the ability of non-metallic materials to enhance microbial growth in drinking water.

a) Method 1 determines the Biomass Production Potential (BPP) by using changes in ATP concentrations as a surrogate measure for active biomass. This method, developed by the Dutch, has been further enhanced as part of the CPDW project 2003 and 2006.

b) Method 2 uses a volumetric measurement of the biofilm. This, German method, was first published as DVGW W 270 in 1984 and is used for certification purposes with limit values established for many years.

c) Method 3 uses dissolved oxygen depletion in water as a surrogate measure of microbial activity (Mean Dissolved Oxygen Difference – MDOD). This British method, first issued as BS DD82 in 1982 and published as BS 6920 Section 2.4 (1988 and 2000), is used for materials approval with limit values.

Each method thus uses different performance characteristics, which allows its use for specific materials or product types but also has limitations. For example, multi-layer pipes cannot currently be tested with the BPP (Method 1) and the MDOD-method (Method 3), and greases or lubricants cannot currently be tested with the BPP (Method 1) and Volumetric-method (Method 2). Harmonised product standards will provide the specific methodology to be followed; this will take into account material of construction and type of components.

All three methods use natural mixtures of aquatic organisms to assess the enhancement of growth by the sample of material. The natural flora comprises many strains that are adapted to living in a relatively hostile environment like drinking water and the results of tests using natural floras have been shown to correlate well with growth on materials in practice. The numbers, types and growth requirements of harmless micro-organisms present in drinking water vary considerably and no single cultural technique exists to enumerate all the aquatic micro-organisms that may be present in a sample of water. Therefore, overall numbers of micro-organisms are generally assessed by using simple indirect measurements of their activity.

The technique for assessing enhanced microbial growth is different in each of the test methods described in this European Standard. In the BPP method described in Method 1 surface and planktonic microbial growth is determined using adenosine triphosphate (ATP) as a surrogate method for active biomass determination. In the Volumetric method (DVGW) described in Method 2, the sum of both active and non-active biofilm on the surface of the test material (living and dead micro-organisms as well as extracellular polymeric substances) is determined volumetrically. In the MDOD method described in Method 3 the measurement of dissolved oxygen uptake is used as a surrogate measure of the growth of both biofilm and planktonic aquatic micro-organisms (most of the organisms which give rise to appreciable microbial growth respire aerobically and exert an influence on the concentration of oxygen dissolved in the water in the test systems).

A variety of factors may influence the capacity of living organisms to respond in a predictable manner and thus validation procedures are an essential part of any biological assay. In all three methods validation is achieved through the use of reference materials.

It is important to note that none of the three methods allows conclusions to be made on the physical (including surface roughness), chemical or toxicological behaviour of materials nor on their resistance to detergents or disinfectants. Additionally, none of the methods provides information on the pathogenicity of any micro-organisms whose numbers may be increased by nutrients leaching from the test material.

WARNING – The tests described in this document should only be carried out in laboratories with suitable facilities and by suitably qualified persons with an appropriate level of chemical and microbiological expertise. Standard microbiological procedures should be followed throughout.

1 Scope

This European Standard specifies three methods for determining the ability of non-metallic materials to enhance the growth of micro-organisms.

This European Standard is applicable to those materials destined to be used under various conditions for the transport and storage of water intended for human consumption.

The standard allows for the testing of a single type of material, or a product in which only one material is in contact with water. It is unsuitable for use with assembled products where more than one material is exposed to water.

NOTE The results given by each method are not directly comparable.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 901, *Chemicals used for treatment of water intended for human consumption — Sodium hypochlorite*

prEN 1254-1:2007, *Copper and copper alloys — Plumbing fittings — Part 1: Fittings with ends for capillary soldering or capillary brazing to copper tubes*

EN 1484, *Water analysis — Guidelines for the determination of total organic carbon (TOC) and dissolved organic carbon (DOC)*

EN 10088-1, *Stainless steels — Part 1: List of stainless steels*

EN 14944-1, *Influence of cementitious products on water intended for human consumption — Test methods — Part 1: Influence of factory made cementitious products on organoleptic parameters*

EN ISO 3696:1995, *Water for analytical laboratory use — Specification and test methods (ISO 3696:1987)*

EN ISO 5814, *Water quality — Determination of dissolved oxygen — Electrochemical probe method (ISO 5814)*

EN ISO 7393-2, *Water quality — Determination of free chlorine and total chlorine — Part 2: Colorimetric method using N, N-diethyl-1, 4-phenylenediamine, for routine control purposes (ISO 7393-2)*

EN ISO 9308-1, *Water quality — Enumeration of Escherichia coli and coliform bacteria — Part 1: Membrane filtration method for waters with low bacterial background flora (ISO 9308-1)*

EN ISO 10012, *Measurement management systems — Requirements for measurement processes and measuring equipment (ISO 10012)*

EN ISO 10523, *Water quality — Determination of pH (ISO 10523)*

EN ISO 13385-1, *Geometrical product specifications (GPS) — Dimensional measuring equipment — Part 1: Callipers; Design and metrological characteristics (ISO 13385-1)*

EN ISO 13385-2, *Geometrical product specifications (GPS) — Dimensional measuring equipment — Part 2: Calliper depth gauges; Design and metrological characteristics (ISO 13385-2)*

EN ISO 16266, *Water quality — Detection and enumeration of Pseudomonas aeruginosa — Method by membrane filtration (ISO 16266)*

ISO 2016, *Capillary solder fittings for copper tubes — Assembly dimensions and tests*

3 Terms and definitions

For the purposes of this document the following terms and definitions apply.

3.1
ATP
adenosine tri-phosphate is the compound produced in the cells of all living organisms which, in the context of this method, is used to measure the amount of active biomass in water and on the surface of a test piece

3.2
Attached Biomass (AB) concentration
micro-organisms producing ATP, attached to a surface

3.3
biofilm
aggregate of micro-organisms that adhere to surfaces in contact with aqueous liquids that are usually embedded in a self-produced matrix of extra-cellular polymeric substances (EPS)

Note 1 to entry: Includes living and non-living organisms

3.4
Biomass Production (BP)
sum of Attached Biomass (3.2) and Suspended Biomass (3.14)

3.5
Biomass Production Potential (BPP)
mean of Biomass Production (3.4) measurements after having taken into account the mean of the growth associated with the negative control

3.6
controls
specific test pieces with known properties with respect to micro biological growth

Note 1 to entry: Controls are used to demonstrate the satisfactory performance of the test

3.7
drinking water
water intended for human consumption conforming to the EC Directive 98/83/EC (on the quality of water intended for human consumption)

3.8
material
formulation of one or more single substances with specified composition and specified production procedure

3.9
negative control
specific test pieces made of materials that are known not to enhance microbial growth

3.10
positive control
specific test pieces made from materials that are known to enhance microbial growth

3.11
product
manufactured item in its finished form

Note 1 to entry: Examples of types of products:

— single material products.

— assembled products. These products comprise two or more components, possibly of different materials.

— multi-layer products. These products comprise two or more layers bonded together to form a single item.

— formulated products. These products are created by chemical processing after which the original constituents are no longer identifiable, e.g. lubricants and glues.

— site applied products. Products such as coatings and linings placed on the market as ingredients that will be mixed and applied on site.

3.12
purified water
purified water complying with Grade 3 of EN ISO 3696:1995

3.13
surface colonisation
micro-organism, attached on any surface in contact with drinking water, which can only be determined by contact cultures, swabs or by other suitable microbiological test procedures

3.14
Suspended Biomass (SB) concentration
micro-organisms producing ATP present in the test water

3.15
test piece
specimen(s) or portion(s) of the test sample which is (are) conditioned, treated or otherwise prepared to be tested to obtain a single test result

3.16
test sample
item(s) drawn from a batch or lot, as received for testing

3.17
test water
drinking water (3.7) together with any additional test method specific requirements

3.18
trace elements
specified substances that need to be present in the test water to ensure consistent microbial growth

4 Influence of materials on water for human consumption – Enhancement of microbial growth (EMG) – Method 1: Measured by Biomass Production Potential (BPP) measured by ATP

4.1 General

The Annexes A to E are a part of Method 1: Measured by (BPP).

This method describes the Biomass Production Potential (BPP) test which determines the growth of micro-organisms in the presence of a material incubated for a specified period of time in biologically stable water.

The Biomass Production Potential (BPP) is a method for testing the ability of a material to promote the growth of microorganisms that is based on a combination of two principles, as follows:

a) batch-based test conditions with replacement of the test water every seven days, and

b) determination of the amount of active biomass by measurement of adenosine tri-phosphate (ATP). Information on ATP and its measurement are given in Annex C.

4.2 Principle

Representative samples of the material to be tested are incubated in appropriately amended drinking water and inoculated with a mixture of naturally occurring micro-organisms derived from a surface source of water. The test pieces are incubated for a specified period of time (16 weeks) at a constant surface area to volume ratio of 0,16 cm^{-1}. This ratio is kept constant adjusting the volume of water in the test containers when a test piece is removed for biomass measurement.

The test water is replaced once every 7 days.

Formation of biomass on the test piece and in the water is determined with adenosine triphosphate (ATP) measurements after 56, 84 and 112 days of incubation. The amount of ATP is used as a measure for the amount of active biomass. The biomass associated with a defined surface area of the test piece is calculated from the amounts of attached and Suspended Biomass.

Each test is validated by the satisfactory performance of the positive and negative controls under equivalent conditions.

The test procedure is shown schematically in Annex E.

4.3 Apparatus

4.3.1 Requirements

Cleaning and sterilisation – all apparatus shall be cleaned and sterilised using procedures that render it suitable for use.

Apparatus and reagents specifically for measurement of ATP are listed separately in Annex B.

4.3.2 Vessels, containers, stoppers and connectors and general laboratory apparatus

Vessels, containers, stoppers and connectors and general laboratory apparatus shall consist of a material, such as glass and stainless steel that is inert under the specified test conditions. PTFE can be used for connectors and stoppers provided that it does not come into contact with the test water. PTFE is unsuitable for larger areas such as containers.

4.3.3 Cleaning

General laboratory glassware, stainless steel plates, test containers and test tubes for dilution water shall be cleaned with a procedure that removes all organic carbon to render it suitable for the requirements of the test.

NOTE A possible way would be by washing with a biodegradable laboratory detergent, followed by rinsing with hydrochloric acid solution, prepared by slowly adding (0,5 ± 0,01) litre of concentrated hydrochloric acid (concentrated, 30 % mass per volume, analytical reagent grade) to (0,5 ± 0,01) litre of purified water and by thoroughly rinsing with purified water. Drain and dry glassware, plates and test containers in a hot air cabinet. Drain the test tubes, cover with caps and heat at 150 °C to 175 °C for 4 h.

4.3.4 Gloves

Gloves for handling test pieces, to avoid contamination from ATP and organic carbon. Powdered latex gloves should be avoided.

4.3.5 Plates

Plates shall be of an appropriate material to be covered completely with the test material. Plates shall be cleaned according to the cleaning procedure described in 4.3.3.

4.3.6 Test containers

Test containers include screw topped glass jars with a volume of 1 000 ml, an internal neck diameter of 57 mm, provided with a lid having a PTFE inlay. Test containers shall be cleaned according to the cleaning procedure described in 4.3.3.

4.3.7 Test tubes

Test tubes shall be sterile glass tubes for preparing dilutions or to contain test pieces during sonication, and fitted with sterile caps. Test tubes shall be cleaned according to the cleaning procedure described in 4.3.3.

4.3.8 Incubator (or hot room)

Incubators shall be capable of maintaining the test temperature of (30 ± 2) °C (in the absence of light), and shall be free from volatile organic compounds.

NOTE Volatile organic compounds in the air cause microbial growth in the test containers, masking the growth attributable to the test pieces.

4.3.9 Sterilisation ovens

Sterilising ovens shall be capable of maintaining temperatures of (160 ± 10) °C, (250 ± 10) °C and (550 ± 20) °C.

4.3.10 Membrane filters

Membrane filters shall have a pore size of 1,2 µm – used for filtering the inoculum (4.4.6).

4.3.11 Autoclave

Autoclave shall be a pressure vessel capable of maintaining (121 ± 3) °C degree Celsius and a gauge pressure of 103 kPa.

4.4 Reagents

4.4.1 Test water

Test water shall be free from harmful effects on bacteria, and meet the quality criteria specified in Table 1. This can be drinking water that meets the additional requirements in Table 1.