INTERNATIONAL STANDARD

ISO 19635

First edition
2016-03-15

Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antialgal activity of semiconducting photocatalytic materials

Céramiques techniques — Méthode d'essai pour l'activité algicide des matériaux photocatalytiques semi-conducteurs

Reference number
ISO 19635:2016(E)

ISO copyright office
Ch. de Blandonnet 8 • CP 401
CH-1214 Vernier, Geneva, Switzerland
Tel. +41 22 749 01 11
Fax +41 22 749 09 47
copyright@iso.org
www.iso.org

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Foreword

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The procedures used to develop this document and those intended for its further maintenance are described in the ISO/IEC Directives, Part 1. In particular the different approval criteria needed for the different types of ISO documents should be noted. This document was drafted in accordance with the editorial rules of the ISO/IEC Directives, Part 2 (see www.iso.org/directives).

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For an explanation on the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the WTO principles in the Technical Barriers to Trade (TBT) see the following URL: Foreword - Supplementary information.

The committee responsible for this document is ISO/TC 206, *Fine ceramics*.

Introduction

Under ultraviolet (UV) light illumination, photocatalysts have diverse functions, such as prevention of fouling, antifogging effects, antibacterial effects, deodorization, and decomposition of air and water contaminants, and their applications have grown in recent years. Since products utilizing these photocatalytic functions are commercialized in large quantities, a method to evaluate and determine photocatalytic effects is required. This International Standard is intended to provide a method for objective evaluation of activity in controlling algae, which are a primary producer of microbial contamination (in the form of environmental biofilms) of outdoor structures, under illumination with ultraviolet light to simulate the outdoor environment and to contribute, via control of algae, to conservation of urban landscapes, prevention of member corrosion, and prevention of fouling of water tank window materials.

Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antialgal activity of semiconducting photocatalytic materials

1 Scope

This International Standard specifies a test method for evaluating anti-algal activities in outdoor structures, specifically flat photocatalytic materials (for example, window panes and water tank glasses, films, guardrails, etc.) under irradiation of ultraviolet light. It does not include powder, granular or porous photocatalytic materials.

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.

ISO 27447, *Fine ceramics (advanced ceramics, advanced technical ceramics) — Test method for antibacterial activity of semiconducting photocatalytic materials*

3 Terms and definitions

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

3.1
photocatalyst
substance with many functions based on oxidization and reduction reactions under *photoirradiation* (3.3), including decomposition and removal of contaminants, deodorization, antibacterial actions, and prevention of fouling

3.2
photocatalytic materials
materials in which or on which the *photocatalyst* (3.1) is added by coating, impregnation, mixing, etc.

Note 1 to entry: Photocatalytic materials are to be used for building and road construction materials to obtain the above-mentioned functions.

3.3
photoirradiation
irradiation of UV light at wavelength 300 nm to 380 nm

3.4
antialgal activity
activity in suppressing growth of algae over a material surface

3.5
photocatalytic antialgal treatment
support of a *photocatalyst* (3.1) by means of various methods, including coating, impregnation, blending, and others, for utilization of the antialgal activity of a photocatalyst

3.6
photocatalytic antialgal activity based on three-point absorbance spectrum method
photocatalytic *antialgal activity* (3.4) derived from the ratio of the absorbance of material treated with *photocatalyst* (3.1) after *photoirradiation* (3.3) of UV light to that of non-treated material after photoirradiation

4 Algae used in the test

The type of algae to be used in the test with photocatalytic-treated antialgal materials shall be *Chlorella vulgaris* (NIES-227). The strain of algae to be used in the test shall be the same strain stored in agencies affiliated with the World Federation for Culture Collections or the Japan Society for Culture Collection.

5 Preparation for the test

5.1 Conditions for handling of algae

The test shall be performed in a laboratory with equipment needed to prevent mixing with other microorganisms.

5.2 Chemicals, materials, and apparatus

Unless otherwise specified, the chemicals, materials, and apparatus to be used in the test shall be as follows. Test tubes, flasks, pipettes, tweezers, etc. shall be carefully cleaned with alkaline or neutral detergent, rinsed thoroughly with water, dried, and sterilized by hot air or high-pressure steam before use.

5.2.1 Purified water, the water used for the preparation of all solutions and culture media and for all determinations shall be distilled or deionized and shall have a conductivity of <1 µS/cm.

5.2.2 Ethanol for disinfection, solution whose ethanol concentration has been adjusted to 77 % to 82 % in volume fraction by means of purified water.

5.2.3 Autoclave, capable of maintaining the temperature at 121 °C ± 1 °C (equivalent to a pressure of 103 kPa).

5.2.4 Dry sterilizer, capable of maintaining the temperature at 160 °C to 180 °C.

5.2.5 Spectrophotometer, capable of measurement within the wavelength range of 400 nm to 800 nm.

5.2.6 Refrigerator, capable of maintaining the temperature at 4 °C ± 1 °C.

5.2.7 Platinum loop, with an end loop of approximately 4 mm.

5.2.8 Glass plate, made from material not affecting the growth of microorganisms.

5.2.9 Cotton plug, plug of non-degreased cotton, or silicon plug, metal plug, or molten plug.

5.2.10 Test tube mixer, for microorganism test.

5.2.11 Contact film, film of material not affecting the growth of microorganisms, which is free of water-absorbing properties and features satisfactory adherence, and in which transmittance in the range of 340 nm to 380 nm, as measured according to the method specified in ISO 27447, accounts for 85 % or more.

5.2.12 Moisture retention glass, glass with thickness of 1,1 mm or less, in which transmittance of 340 nm to 380 nm, as measured according to the method specified in ISO 27447, accounts for 85 % or more and which has been cut to a size capable of covering the entire surface of a Petri dish.

5.2.13 Storage Petri dish, dish with the inside diameter of about 90 mm.

5.2.14 Moisture-conditioning filter paper, filter paper not affecting the growth of microorganisms, which has been cut to a size appropriate for placement in the container in which the test piece is to be set.

5.2.15 Disposable cuvette, plastic cuvettes (about 12 mm × 12 mm × 45 mm) appropriate for placement in the storage container.

5.2.16 Tooth brush, soft type of brush made of polyamide.

5.2.17 UV fluorescent lamp, 20-W black-light blue type UV fluorescent lamp (BLB lamp) that provides UV-A ranging from 300 nm to 400 nm with a peak emission at 351 nm.

5.2.18 Fluorescent lamp, 20-W starter and straight-type white (symbol: W) lamp.

5.2.19 UV irradiator, system comprised of one UV fluorescent lamp, which can shield light from the surrounding region.

5.2.20 UV light radiometer, radiometer with a detector whose sensitivity peak is at 351 nm and provide limit, e.g. ±10 nm, shall be used. The radiometer shall be calibrated for the light source to be used or corrected to ascertain sensitivity within the wavelength range to be absorbed by the photocatalytic test piece with suitable approach.

5.2.21 Illuminometer, general type AA-grade illuminometer.

5.2.22 Filter unit, in which the membrane filter is made from a less-absorbent material, such as hydrophilic polyvinylidene fluoride, hydrophilic polytetrafluoroethylene, etc., and the filter pore size is 0,22 µm or 0,45 µm.

5.2.23 Centrifuge, capable of the centrifugal force to 1 500 × g for preparation of test solutions.

5.3 Sterilization and filtration methods

5.3.1 Hot-air sterilization

The apparatus to be sterilized shall be placed in a hot-air sterilizer at 160 °C to 180 °C and held at this temperature for 30 min to 60 min. If the sterilized sample exhibits any wet cotton plug or package water after completion of hot-air sterilization, the apparatus shall not be used.

5.3.2 Hot-steam sterilization

An autoclave shall be filled with water and the apparatus to be sterilized shall be placed in a wire cage and placed on the autoclave shelf. The autoclave shall be closed with the lid and heated and held at a temperature of 121 °C (equivalent to a pressure of 103 kPa) for 15 min to 20 min. After discontinuing heating and allowing the temperature to cool to 100 °C or less, the exhaust valve shall be opened to bleed steam and the lid shall be opened. The apparatus sterilized in this fashion shall be taken out and, if necessary, allowed to cool in the safety cabinet.

In order to maintain cleanness to prevent contamination by culture media and processing chemicals, the autoclave shall be cleaned with neutral detergent as required and rinsed with water thoroughly.

5.3.3 Flaming

The apparatus to be sterilized shall be subjected to gas or alcohol flame treatment. Platinum loops shall be subjected to flame treatment until they become red hot, while test tubes shall be subjected to flame treatment for two to three seconds.

5.3.4 Sterilization with alcohol

Absorbent cotton or gauze shall be soaked with ethanol for disinfection and squeezed lightly, for use in wiping both hands.

5.3.5 Sterilization through filtration

Liquid to be sterilized shall be filtered with the filter unit.

5.4 Culture media

5.4.1 General

Culture media to be used shall be of the composition shown in Table 1 to Table 3.

Table 1 — C culture medium

Tris (hydroxymethyl) aminomethane	500 mg
$Ca(NO_3)_2 \cdot 4H_2O$	150 mg
KNO_3	100 mg
Glyceric acid disodium 5.5 hydrate	50 mg
$MgSO_4 \cdot 7H_2O$	40 mg
PIV metallic salt solution	3 ml
Cyanocobalamin (vitamin B_{12}) solution	1 ml
Biotin solution	1 ml
Thiamine hydrochloride (vitamin B_1) solution	1 ml
Vitamin mixture	0,1 ml
Distilled water (purified water)	994 ml

The contents shall be thoroughly dissolved.

Table 2 — PIV metallic salt solution

Ethylene diamine tetra-acetic acid dihydrogen disodium dihydrate (EDTA2Na·$2H_2O$)	1 000 mg
$FeCl_3 \cdot 6H_2O$	196 mg
$MnCl_2 \cdot 4H_2O$	36 mg
$ZnSO_4 \cdot 7H_2O$	22 mg
$CoCl_2 \cdot 6H_2O$	4,0 mg
$Na_2MoO_4 \cdot 2H_2O$	2,5 mg
Distilled water (purified water)	1 000 ml

Table 3 — Vitamin solutions

Cyanocobalamin (vitamin B_{12}) solution	1,0 mg/l
Biotin solution	1,0 mg/l
Thiamine hydrochloride (vitamin B_1) solution	100 mg/l

As for the PIV metallic salt and vitamin solutions, after thorough dissolution of the contents and subsequent sterilization through filtration, the required quantity shall be dispensed into a sterilized bottle, which shall then be plugged tightly and stored in a dark place at 4 °C. The stored sample shall be used within three months.

5.4.2 C culture medium

After thorough dissolution, the pH shall be adjusted to pH 7,5 with HCl. Finally, sterilization shall be performed by the autoclaving.

5.4.3 Slant medium

In 1 000 ml of the C culture medium, 15,0 g of agar powder shall be mixed. The contents shall be allowed to dissolve thoroughly in a boiling water bath (C agar culture medium). Approximately 10 ml of dissolved C agar culture medium shall be poured into the test tube, which shall then be plugged with a cotton plug and subjected to sterilization with high-pressure steam. After completion of sterilization, the test tube shall be left inclined at about 15° to 30° relative to the horizontal surface in the clean room. The content allowed to solidify in this state is called slant medium.

The slant medium may also be prepared by pouring the high-pressure steam-sterilized C agar culture medium into the test tube previously sterilized with hot air and by allowing the content thus prepared to solidify in the inclined test tube as described above.

5.4.4 Washing solution

Prepared by adding and dissolving 3,04 g of $NaH_2PO_4 \cdot 2H_2O$ and 10,92 g of $Na_2HPO_4 \cdot H_2O$ in 1 000 ml of purified water, followed by sterilization with high-pressure steam.

6 Cultivation of algae

6.1 Transplantation and cultivation of algae

The *Chlorella* to be tested shall be transplanted aseptically in the clean bench or in an environment with cleanliness equivalent to that of the clean room. The test tube for base strain and that into which transplantation is to be performed shall have their cotton plug and neck wiped with absorbed cotton or gauze soaked with ethanol for disinfection and then set in the tube rack and placed in the clean bench. The test tube with base strain and that with slant medium to which the sample *Chlorella* is to be transplanted shall be held in one hand and the platinum loop in the other. Cotton plugs shall be removed with the other hand holding the loop, with the mouth of test tube sterilized by flame. Then, the platinum loop shall be sterilized by flame and inserted into a slant medium portion containing condensed water for thorough cooling. The cooled platinum loop shall be placed in the test tube for base strain to scrape off one loopful from the *Chlorella* growth surface and this amount sprayed into the new slant medium. The mouth of the test tube shall be sterilized again by flame and plugged by the cotton plug as originally. After use, the platinum loop shall be sterilized by frame. The slant medium to which *Chlorella* has been transplanted shall be cultivated at a temperature of 25 °C ± 1 °C and under visible light at an intensity of 5 µmol/m^2s to 10 µmol/m^2s (about 500 lx to 1 000 lx). When one loopful of *Chlorella* is to be sprayed over the slant medium, *Chlorella* shall be dispersed in condensed water as shown in Figure 1 and a straight line shall be drawn from there upward along the slope. An end of the platinum loop shall be lifted temporarily from the medium, immersed again in condensed air, and shall this time draw a meandering line upward along the slope. Do not use the *Chlorella* cultivated on slant medium for over three months.

NOTE See Figure 1.