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

Céramiques techniques — Méthode d'essai de l'activité antibactérienne des matériaux photocatalytiques semiconducteurs

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Foreword

ISO (the International Organization for Standardization) is a worldwide federation of national standards bodies (ISO member bodies). The work of preparing International Standards is normally carried out through ISO technical committees. Each member body interested in a subject for which a technical committee has been established has the right to be represented on that committee. International organizations, governmental and non-governmental, in liaison with ISO, also take part in the work. ISO collaborates closely with the International Electrotechnical Commission (IEC) on all matters of electrotechnical standardization.

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).

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights. Details of any patent rights identified during the development of the document will be in the Introduction and/or on the ISO list of patent declarations received (see www.iso.org/patents).

Any trade name used in this document is information given for the convenience of users and does not constitute an endorsement.

For an explanation of the voluntary nature of standards, the meaning of ISO specific terms and expressions related to conformity assessment, as well as information about ISO's adherence to the World Trade Organization (WTO) principles in the Technical Barriers to Trade (TBT), see www.iso.org/iso/foreword.html.

This document was prepared by Technical Committee ISO/TC 206, *Fine ceramics*. This second edition cancels and replaces the first edition (ISO 27447:2009), which has been technically revised. The main changes to the previous edition are as follows:

— updating of reference document and cross-references;

— replacement of “adhesive” with “cover” throughout;

— clarification of definition of “photocatalyst antibacterial activity value” (3.4, 3.5, 3.6, 3.7) in Clause 3;

— deletion of a definition of “fluorescent UV lamp” in Clause 3 due to updating of the reference document ISO 10677;

— inclusion of a statement in Clause 5 regarding treatment of results measured by the viable bacterial count method;

— NOTE 1 changed to body text in 6.1.3;

— revision of “storage period of 1/500 nutrient broth” from 1 month ago to 1 week ago in 6.2.2 (formerly 6.2.1);

— addition of a new subclause, 6.2.1, renumbering of subsequent subclauses and updating of cross-references in Clause 6;

— addition of a new subclause, 7.1, and renumbering of subsequent subclauses in Clause 7;

— revision of Figure 1, Figure 4 and Figure 5;

— addition of “paper filter” apparatus in 7.6;

— replacement of “black light fluorescent lamp” with “light source” in 7.7 (formerly 7.5) and revision of a statement in 7.7 that the light source shall be 351BLB or 351BL as specified in ISO 10677;

— replacement of “ultraviolet light radiation mater” with “UV radiometer” in 7.8 (formerly 7.6) and inclusion of a statement in 7.8 that the UV radiometer shall be used as specified in ISO 10677;

— NOTE changed to body text in 8.1;

— addition of a new subclause, 9.1, and renumbering of subsequent subclauses in Clause 9;

— revision of storage time of “the bacteria suspension in case of not using immediately” from 4 h to 2 h in 9.2.1(formerly 9.1.1);

— NOTE 2 changed to body text in 9.2.2 (formerly 9.1.2);

— NOTE 2 and NOTE 3 changed to body text in 9.3.2 (formerly 9.2.2);

— addition of the test environment temperature (25 °C ± 3 °C) in 9.4.1;

— addition of a new subclause, 10.1, and renumbering of subsequent subclauses in Clause 10.

Any feedback or questions on this document should be directed to the user’s national standards body. A complete listing of these bodies can be found at www.iso.org/members.html.

Introduction

This document was developed for antibacterial activity as a result of continuing efforts to provide test methods for photocatalytic materials. However, antibacterial activity cannot be measured for test pieces with permeable or rough surfaces, so other test methods are required.

Under the irradiation of photons, photocatalysts show diverse functions, such as the decomposition of air and water contaminants, as well as deodorization, self-cleaning, antifogging and antibacterial actions. These functions of photocatalysts are generally based on the action of active oxygen species such as hydroxyl (OH) radicals formed on the surface of a photocatalyst (References [14] and [15]). The energy- and labour-saving nature of photocatalysis has attracted keen interest when the photocatalyst is activated by sunlight (or artificial lighting).

Practical applications of photocatalysts for both indoor and outdoor use have rapidly expanded in recent years. Many kinds of photocatalytic materials have been proposed or are already commercialized, based on ceramics, glass, concrete, plastics or paper. Such materials are produced by either the coating or mixing of a photocatalyst; in most cases, titanium dioxide (TiO_2).

However, the effect of photocatalysis is not easily inspected visually, and no appropriate or official evaluation methods have been available to date. Some confusion has thus arisen as photocatalytic materials have been introduced. Furthermore, the above-mentioned diverse functions of photocatalysts cannot be evaluated with a single method; thus, it is necessary to provide different evaluation methods for air purification, water decontamination and self-cleaning.

This document applies to the testing of the antibacterial activity of photocatalytic ceramics and other materials produced by either the coating or the mixing of a photocatalyst. Standards for testing the antifungal activity that use photocatalytic materials will be developed separately.

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WARNING — Handling and manipulation of microorganisms that are potentially hazardous requires a high degree of technical competence. Only personnel trained in microbiological techniques should carry out tests.

1 Scope

This document specifies a test method for the determination of the antibacterial activity of materials that contain a photocatalyst or have photocatalytic films on the surface, by measuring the enumeration of bacteria under irradiation of ultraviolet light.

This document is intended for use with different kinds of semiconducting photocatalytic materials used in construction materials in flat sheet, board, plate shape or textiles that are the basic forms of materials for various applications. It does not include powder, granular or porous photocatalytic materials.

This test method is usually applicable to photocatalytic materials produced for antibacterial effect. Other types of performance of photocatalytic materials, i.e. antifungal activity, antiviral activity, decomposition of water contaminants, self-cleaning, antifogging and air purification, are not determined by this method.

The values expressed in this document are in accordance with the International System of Units (SI).

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements 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.

ISO 10677, *Fine ceramics (advanced ceramics, advanced technical ceramics) — Ultraviolet light source for testing semiconducting photocatalytic materials*

ISO 80000-1, *Quantities and units — Part 1: General*

3 Terms and definitions

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

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

— ISO Online browsing platform: available at https://www.iso.org/obp

— IEC Electropedia: available at http://www.electropedia.org/

3.1
photocatalyst
substance that carries out many functions based on oxidization and reduction reactions under ultraviolet (UV) irradiation, including decomposition and removal of air and water contaminants, deodorization, and antibacterial, antifungal, antiviral, self-cleaning and antifogging actions

3.2
photocatalytic material
material in which or on which the photocatalyst is added by coating, impregnation or mixing

Note 1 to entry: Photocatalytic materials are to be used for building and road construction materials to obtain the functions mentioned in 3.1.

3.3
antibacterial
condition inhibiting the growth of bacteria on the surface of flat surface materials or cloths

3.4
photocatalyst antibacterial activity value for film cover method
difference between the logarithms of the total number of viable bacteria on photocatalytic treated materials after UV irradiation and on non-treated materials after UV irradiation

Note 1 to entry: This value includes the decrease in the number of bacteria without UV irradiation.

3.5
photocatalyst antibacterial activity value for glass cover method
difference between the logarithms of the total number of viable bacteria on photocatalytic treated cloths after UV irradiation and on standard cloths after UV irradiation

Note 1 to entry: This value includes the decrease in the number of bacteria without UV irradiation.

3.6
photocatalyst antibacterial activity value with UV irradiation for film cover method
difference between the logarithms of the total number of viable bacteria on photocatalytic treated materials after UV irradiation and on photocatalytic treated materials kept in a dark place

3.7
photocatalyst antibacterial activity value with UV irradiation for glass cover method
difference between the logarithms of the total number of viable bacteria on photocatalytic treated cloths after UV irradiation and on photocatalytic treated cloths kept in a dark place

3.8
film cover method
test method to evaluate the antibacterial performance of photocatalytic flat surface materials

3.9
glass cover method
test method to evaluate antibacterial performance of photocatalytic cloths

4 Symbols

A average number of viable bacteria on non-treated specimens, just after inoculation

B_D average number of viable bacteria on non-treated specimens, after being kept in a dark place

B_L average number of viable bacteria on non-treated specimens, after UV irradiation of intensity L

C_D average number of viable bacteria on photocatalytic treated specimens, after being kept in a dark place

C_L average number of viable bacteria on photocatalytic treated specimens, after UV irradiation of intensity L

F_{BD} growth value, after being kept in a dark place

F_{BL} growth value, after UV irradiation of intensity L

L UV irradiation intensity

L_{max} maximum logarithmic value of viable bacteria

L_{mean} average logarithmic value of viable bacteria for three specimens

L_{min} minimum logarithmic value of viable bacteria

M number of viable bacteria with glass cover method

M_{BA} average logarithmic value of the number of viable bacteria for three non-treated specimens, just after inoculation

M_{BD} average logarithmic value of the number of viable bacteria for three non-treated specimens, after being kept in a dark place

M_{BL} average logarithmic value of the number of viable bacteria for three non-treated specimens, after UV irradiation of intensity L

M_D average logarithmic value of the number of viable bacteria for three photocatalytic treated specimens, after being kept in a dark place

M_L average logarithmic value of the number of viable bacteria for three photocatalytic treated specimens, after UV irradiation of intensity L

N number of viable bacteria with film cover method

P bacteria concentration

D_F dilution factor

R_L photocatalyst antibacterial activity value for film cover method, after irradiation at a constant intensity L

ΔR photocatalyst antibacterial activity value with UV irradiation for film cover method

S_L photocatalyst antibacterial activity value for glass cover method, after UV irradiation of intensity L

ΔS photocatalyst antibacterial activity value with UV irradiation for glass cover method

V volume of soybean casein digest broth with lecithin and polysorbate 80 medium for washout

Z average number of colonies in two Petri dishes

5 Principle

This document is for the development, comparison, quality assurance, characterization, reliability and design data generation of photocatalytic materials. The method is used to obtain the antibacterial activity of photocatalytic materials by the contact of a specimen with bacteria, under UV light irradiation. The film cover method is available for flat sheet, board or plate-shaped materials. To avoid warpage in the cloths or textiles, the glass cover method is available for the cloths or textiles.

The specimen is laid in a Petri dish and the bacterial suspension is dripped onto the specimen. Then the cover film or glass is placed on the suspension and the moisture conservation glass is placed on top of the Petri dish. The Petri dish containing the specimen is exposed to light. After exposure, the test bacteria are washed out of the specimen and the cover film or glass. This washout suspension is measured by the viable bacterial count method. The results obtained are compared with those obtained

from inoculated specimens of non-photocatalytic treated material exposed to UV irradiation under identical conditions to the treated material, and to those obtained from inoculated specimens of both photocatalytic treated and non-treated material kept in the dark for the same period of time.

6 Materials

6.1 Bacteria used and preparation for tests

6.1.1 Film cover method

a) *Staphylococcus aureus*

b) *Escherichia coli*

6.1.2 Glass cover method

a) *Staphylococcus aureus*

b) *Klebsiella pneumoniae*

6.1.3 Bacteria preparation

The bacteria strains to be used in the test are equivalent to those described in Table 1 and are stored by entities that are registered under the World Federation for Culture Collections or the Japan Society for Culture Collections.

Aseptic manipulations using microorganisms can be performed in an adequate safety cabinet. Inoculate each strain into a slant culture medium (nutrient agar medium), incubate for 16 h to 24 h at 37 °C ± 1 °C, and then store in a refrigerator at 5 °C to 10 °C. Repeat subcultures within 1 month by replicating this process. The maximum number of subcultures from the original strain transferred by culture collection is 10 times. In the case of bacteria stored in a deep freezer, the maximum number of subcultures from the original strain transferred by culture collection is 10. The slant culture shall not be used for further storing after 1 month.

NOTE If necessary, additional tests with other bacteria can be carried out.

Table 1 — Bacteria strains to be used in test

Bacteria species	Strain number	Organization for the collection
Staphylococcus aureus	ATCC 6538P	American Type Culture Collection
	DSM 346	German Collection of Microorganisms and Cell Cultures (DSMZ)
	NBRC 12732	NITE Biological Resource Center
Escherichia coli	ATCC 8739	American Type Culture Collection
	DSM 1576	German Collection of Microorganisms and Cell Cultures (DSMZ)
	NBRC 3972	NITE Biological Resource Center
Klebsiella pneumoniae	ATCC 4352	American Type Culture Collection
	DSM 789	German Collection of Microorganisms and Cell Cultures (DSMZ)
	NBRC 13277	NITE Biological Resource Center