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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.

International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

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.

ISO 27447 was prepared by Technical Committee ISO/TC 206, *Fine ceramics*.

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Introduction

As a result of continuing efforts to provide test methods for photocatalytic materials, this International Standard was developed for antibacterial activity. However, for test pieces with permeable or rough surfaces, etc., the antibacterial activity cannot be measured, 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 the photocatalyst (References [10] and [11] in the Bibliography). 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, paper, etc. 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 and 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 required to provide different evaluation methods for air purification, water decontamination and self-cleaning.

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This International Standard applies to testing the antibacterial activity of photocatalytic ceramics and other materials produced by either the coating or 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 International Standard 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 International Standard 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 an antibacterial effect. Other types of performance of photocatalytic materials, i.e. decomposition of water contaminants, self-cleaning, antifogging and air purification, are not determined by this method.

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

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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.

ISO 31-0, *Quantities and units — Part 0: General principles*

3 Terms and definitions

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

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, self-cleaning and antifogging actions

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

NOTE Photocatalytic materials are to be used for building and road construction materials to obtain the functions mentioned in 3.1.

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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 adhesion method
difference between the total number of viable bacteria of photocatalytic treated flat surface materials and non-treated materials after UV irradiation

NOTE This value includes the decrease of the number of bacteria without UV irradiation.

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

NOTE This value includes the decrease of the number of bacteria without UV irradiation.

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

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

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

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3.9
glass adhesion method
test method to evaluate the antibacterial performance of photocatalytic cloths

3.10
fluorescent UV lamp
lamp that provides UV-A irradiation within a wavelength range of 300 nm to 400 nm

NOTE A suitable lamp is the so-called black light blue (BLB) fluorescent lamp, with a maximum at 351 nm, as described in ISO 4892-3.

4 Symbols

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

B_{D} average number of viable bacteria of non-treated specimens, after being kept in a dark place

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

C_{D} average number of viable bacteria of photocatalytic treated specimens, after being kept in a dark place

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

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

$F_{\mathrm{B}L}$ growth value, after UV irradiation of intensity L

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L UV irradiation intensity

L_{max} maximum logarithmic value of viable bacteria

L_{mean} average logarithmic value of viable bacteria for 3 specimens

L_{min} minimum logarithmic value of viable bacteria

M number of viable bacteria with glass adhesion method

M_{BA} average logarithmic value of the number of viable bacteria for 3 non-treated specimens, just after inoculation

M_{BD} average logarithmic value of the number of viable bacteria for 3 non-treated specimens, after being kept in a dark place

M_{BL} average logarithmic value of the number of viable bacteria for 3 non-treated specimens, after UV irradiation of intensity L

M_{D} average logarithmic value of the number of viable bacteria for 3 photocatalytic treated specimens, after being kept in a dark place

M_{L} average logarithmic value of the number of viable bacteria for 3 photocatalytic treated specimens, after UV irradiation of intensity L

N number of viable bacteria with film adhesion method

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P bacteria concentration

R dilution factor

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R_{L} photocatalyst antibacterial activity value, after irradiation at a constant intensity (L) on a photocatalytic material

ΔR photocatalyst antibacterial activity value with UV irradiation

S_{L} photocatalyst antibacterial activity value, after UV irradiation of intensity L

ΔS photocatalyst antibacterial activity value with UV irradiation

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

Z average number of colonies in 2 Petri dishes

5 Principle

This International Standard 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 adhesion method is available for flat sheet, board or plate-shaped materials. To avoid warpage in the cloths or textiles, the glass adhesion method is available for cloths or textiles.

The specimen is laid in a Petri dish and the bacterial suspension is dripped onto the specimen. Then the adhesive 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 adhesive film or glass. This washout suspension is measured by the viable bacterial count method.

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6 Materials

6.1 Bacteria used and preparation for tests

6.1.1 Film adhesion method

a) *Staphylococcus aureus*

b) *Escherichia coli*

6.1.2 Glass adhesion 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. The slant culture must not be used for further storage after 1 month.

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NOTE 1 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.

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NOTE 2 If necessary, additional tests with other bacteria can be allowed.

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

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6.2 Chemicals and implements

6.2.1 1/500 nutrient broth

For 1 000 ml of purified water, take 3,0 g of meat extract, 10,0 g of peptone and 5,0 g of sodium chloride, put them in a flask and dissolve them thoroughly. When the contents are thoroughly diluted, use a solution of sodium hydroxide or hydrochloric acid to bring the pH to (7,1 ± 0,1) at 25 °C. Dilute this medium 500 times using purified water, and set the pH to (7,0 ± 0,2) using hydrochloric acid solution or sodium hydroxide solution. Sterilize in an autoclave at 121 °C ± 2 °C for at least 15 min. After preparation, if 1/500 nutrient broth is not used immediately, store it at 5 °C to 10 °C. Do not use 1/500 nutrient broth made more than 1 month ago.

6.2.2 Nutrient broth

For 1 000 ml of purified water, take 3,0 g of meat extract, 10,0 g of peptone and 5,0 g of sodium chloride, put them in a flask and dissolve them thoroughly. When the contents are thoroughly diluted, use a solution of sodium hydroxide or hydrochloric acid to bring the pH to (7,1 ± 0,1) at 25 °C. If necessary, dispense the contents in a test tube, add a cotton plug and sterilize in an autoclave (see 6.2.1). After preparation, if nutrient broth is not used immediately, store it at 5 °C to 10 °C. Do not use nutrient broth made more than 1 month ago.

6.2.3 Nutrient agar

For 1 000 ml of purified water, take 3,0 g of meat extract, 5,0 g of peptone and 15,0 g of agar powder, put them in a flask and mix. Heat the flask in boiling water to dissolve the contents thoroughly. Use a 0,1 mol/l solution of sodium hydroxide to bring the pH to (6,8 ± 0,2) at 25 °C. Add a cotton plug and sterilize in an autoclave (see 6.2.1). After preparation, if nutrient agar is not used immediately, store it at 5 °C to 10 °C. Do not use nutrient agar made more than 1 month ago. Keep the medium temperature between 45 °C and 48 °C when mixing with a bacterial suspension.

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6.2.4 Soybean-casein digest broth with lecithin and polysorbate 80 (SCDLP)

For 1 000 ml of purified water, take 17,0 g of casein peptone, 3,0 g of soybean peptone, 5,0 g of sodium chloride, 2,5 g of phosphoric acid monopotassium dehydrogenate, 2,5 g of glucose and 1,0 g of lecithin, put them in a flask and dissolve them. Add 7,0 g of non-ionic surfactant and dissolve it. Use a solution of sodium hydroxide or hydrochloric acid to bring the pH to (7,0 ± 0,2) at 25 °C. If necessary, dispense it in a test tube, add a cotton plug and sterilize in an autoclave (see 6.2.1). After preparation, if SCDLP is not used immediately, store it at 5 °C to 10 °C. Do not use SCDLP medium made more than 1 month ago.

6.2.5 Physiological saline solution

For 1 000 ml of purified water, take 8,5 g of sodium chloride, put it in a flask and dissolve it thoroughly. If necessary, dispense it in a test tube and sterilize in an autoclave (see 6.2.1). After preparation, if physiological saline solution is not used immediately, store it at 5 °C to 10 °C. Do not use physiological saline solution made more than 1 month ago.

6.2.6 Physiological saline solution for washout

For 1 000 ml of purified water, take 8,5 g of sodium chloride, put it in a flask and dissolve it thoroughly. Add 2,0 g of non-ionic surfactant and dilute. If necessary, dispense 20 ml of the solution in a test tube or Erlenmeyer flask and sterilize in an autoclave (see 6.2.1). After preparation, if physiological saline solution for washout is not used immediately, store it at 5 °C to 10 °C. Do not use physiological saline solution made more than 1 month ago for washout.

6.2.7 Non-ionic surfactant

Polyoxyethylene sorbitan monooleate (polysorbate 80).

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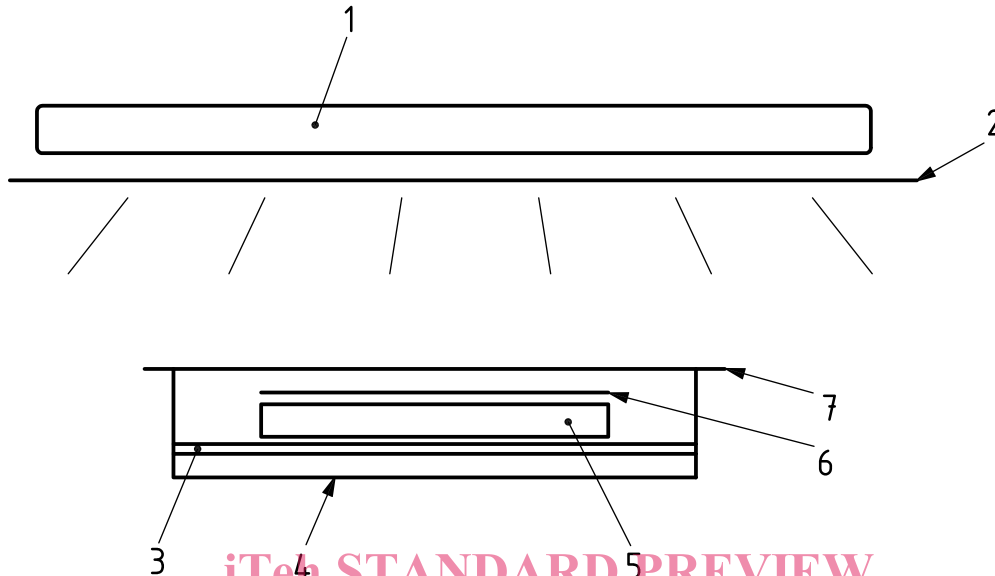
7 Apparatus

The test equipment enables a photocatalytic material to be examined for its antibacterial activity by providing UV irradiation to activate the photocatalyst. It consists of a light source and a chamber with a test piece. An example of a test system is shown in Figure 1.



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Key

1 light source
2 punched metal
3 glass stick
4 paper filter
5 test piece
6 adhesive film or glass
7 moisture preservation glass

Figure 1 — Schematic diagram of the test equipment

7.1 Adhesive film

The adhesive film is inert and non-water absorbent with good sealing properties, with a transparency rate over 85 % for the 340 nm to 380 nm range. The sheets are cut with dimensions of (40 ± 2) mm.

NOTE Reference data for adhesive films is given in Annex B.

7.2 Adhesive glass

The adhesive glass consists of glass panes with a thickness less than or equal to 1,1 mm, with a transparency rate over 85 % for the 340 nm to 380 nm range. The panes are cut with dimensions of (40 ± 2) mm.

NOTE Reference data for adhesive glasses is given in Annex B.

7.3 Moisture preservation glass

The moisture preservation glass consists of glass panes with a thickness less than or equal to 1,1 mm, with a transparency rate over 85 % for the 340 nm to 380 nm range. The panes are cut to fully cover Petri dishes.

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