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Fine bubble technology — Water treatment applications —

Part 2: Test methods using *Escherichia coli* as a test micro-organism

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Foreword

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This document was prepared by Technical Committee ISO/TC 281, *Fine bubble technology*.

A list of all parts in the ISO 20304 series can be found on the ISO website.

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

In the last decade, fine bubble technology generated by hydrodynamic cavitation methods has been applied as part of water treatment facilities for water disinfection purposes. However, absence of International Standards in relation to the efficiency of water disinfectant properties on fine bubbles has been an obstacle to trading and technology transfer. This document describes a method for the determination of water disinfection efficiency of fine bubbles generated by a hydrodynamic cavitation.

Fine bubbles act as a disinfectant through chemical (i.e. generation of OH radicals) and physical mechanisms caused by presence of shock waves, pressure gradients and shear forces. The function as a disinfectant of fine bubbles was also found in that fine bubbles provide a more effective means for cleaning and disinfecting both, the bath and the reservoir, than traditional ultrasonic vibrator.[1]

Properties of fine bubbles and their disinfection mechanisms differ according to applied generation processes, bubble size distributions and bubble densities. However existing studies showed that fine bubbles generated have a significant water disinfection effect. Particularly, high deactivation efficiency of *Escherichia coli* (*E. coli*) has been achieved in water disinfection by microbubbles generated using hydrodynamic cavitation as well.[2]-[4]

Generation of highly reactive free radicals and turbulence associated with collapsing micro bubbles provides great potential for water disinfection. The effect of ozone microbubble on *E. coli* has often been found more effective and with faster disinfection kinetics of *E. coli*, when the ozone gas is activated as the micro or ultrafine bubble forms.[5],[6]

The test methods on the antibacterial activity of textile products have been standardized by ISO/TC 38, *textiles*. The present standard operation procedure (SOP) for the evaluation of disinfection efficiency is based on the quantitative measurement by ATP luminescence method for the evaluation of antibacterial activity.

Therefore, under the test conditions, establishment of International Standards to measure the water disinfection efficiency of fine bubbles is essential to promote the relevant trading technology transfer.

Fine bubble technology — Water treatment applications —

Part 2:
Test methods using *Escherichia coli* as a test micro-organism

1 Scope

This document specifies a test method for assessing bactericidal viability of *Escherichia coli* as a test micro-organism, in dispersions of various fine bubbles generated by the hydrodynamic cavitation of water medium.

2 Normative references

There are no normative references in this document.

3 Terms and definitions

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

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

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

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

3.1
hydrodynamic cavitation
fine bubble generation method used by physical hydrodynamic structures and pressures

3.2
disinfection efficiency
ratio of viable cell count to the initial number of the test bacteria

3.3
control suspension
suspension used to validate the growth condition of test bacteria and validate the test, i.e. bacterial test suspension produced without using fine bubble generation

3.4
test solution
solution samples obtained for the analysis of antibacterial activity after operating the test facility

3.5
test gas
gas mixed in the middle of operating the cavitation unit

3.6
relative light unit
RLU
unit used for adenosine triphosphate (ATP) luminescence measurement

4 Safety precautions

The test methods specified in this document require the use of bacteria and conditions that promote bacterial growth. Since the bacteria can be pathogenic, the tests should be carried out by persons with training and experience in the use of microbiological techniques. The laboratory facilities should be designated as appropriate biosafety level.[7]

Appropriate safety precautions should be observed with due consideration given to country-specific.

5 Requirements

This document is to provide the measurement method of disinfection efficiency for fine bubbles enhanced waters by test hydrodynamic cavitation units. The disinfection efficiency is measured by the test fine bubble solution generated by each test hydrodynamic cavitation unit.

The antibacterial (bactericidal) activity shall be evaluated using the following test organism: *E. coli* (see Table A.1). The test tank and test facility should be cleaned by appropriate methods like triple-wash with sterilized water after chlorination before the test.

6 Test methods

6.1 Principle

The test tank is filled with a test suspension of bacteria and the test hydrodynamic cavitation (HC) unit is operated under the required initial test condition (approximately 15 °C to 24 °C ± 1 °C). The fine bubbles-enriched water will be contacted to the test suspension of bacteria in the test tank (see Figure 1).

At a specific contact time, an aliquot is taken; the bactericidal action in this portion is immediately measured using the luminescence photometer (see 6.4.8). First, the relationship between the measurement unit of the luminescence photometer, relative light unit (RLU) (see Annex C) and counted colonies forming unit (CFU) of the initial density of the test microorganism is measured to check the initial cell density of the bacterial test suspension.

The test is performed using *E. coli* (see Table A.1).

6.2 Test facility

Fine bubbles are generated by circulating the liquid in a fine bubble generating system with the following characteristics: a 125-litre tank, a 0,4 kW centrifugal multistage pump made of stainless steel, PVC pipes and a cavitation unit with the test fine bubble generating unit (see Figure 1). Test gas can be chosen for the appropriate test purposes.

6.3 Culture media and reagents

6.3.1 General

The reagents shall be of analytical grade and/or appropriate for microbiological purposes.

To improve reproducibility, dehydrated material should be used for the preparation of culture media. The manufacturer's instructions relating to the preparation of these products should be rigorously followed.

6.3.2 Water

Water used in tests shall be analytical-grade water for microbiological media preparation, which is freshly distilled and/or ion-exchanged and/or ultra-filtered and/or filtered with reverse osmosis (RO). It shall be free from substances that are toxic or inhibiting to the bacteria.

6.3.3 Tryptone soya agar (TSA)

TSA used in the test is for maintenance of bacterial strains and performance of viable counts.

Tryptone, pancreatic digest of casein	15,0 g
Soya peptone, papaic digest of soybean meal	5,0 g
NaCl	5,0 g
Agar	5,0 g
Water (see 6.3.2)	to 1,0 l

Sterilize in the autoclave (6.4.1). After sterilization, the pH of the medium shall be 7,2 ± 0,2 when measured at 20 °C.

6.3.4 Tryptone soya broth (TSB)

Tryptone, pancreatic digest of casein	17 g
Soya peptone, papaic digest of soya	3 g
Sodium chloride (NaCl)	5 g
Glucose	2,5 g
Dipotassium hydrogen phosphate	2,5 g
Water	1 000 ml

Mix well and adjust pH, 7,2 ± 0,2, then sterilize by autoclave (6.4.1).

6.3.5 Lysogeny broth (LB)

Tryptone, pancreatic digest of casein	10 g
Yeast extract	5 g
NaCl	10 g
Water	1 000 ml

Mix well and adjust pH, 6,9 ± 0,2, then sterilize by autoclave (6.4.1).

6.3.6 Cryoprotective solution for bacterial species

For freezing, a cryoprotective solution containing 150 g/l of glycerol or 100 g/l of dimethylsulfoxide shall be used and prepared as follows:

TSB (see 6.3.4) or LB (see 6.3.5):	1 000 ml
Add Glycerol: 150 g or dimethylsulfoxide:	100 g

Mix well and sterilize by autoclave (6.4.1).

For solutions containing glycerol, sterilize the mixed solution by autoclave (6.4.1). For solutions containing dimethylsulfoxide, sterilize the mixed solution by using a 0,22 µm membrane filter.

NOTE Any commercially available product can be used as long as it is a cryoprotective solution or preserving system that contains glycerol or dimethylsulfoxide.

6.3.7 Physiological saline

Sodium chloride (NaCl)	8,5 g
Water	1 000 ml

Mix well, then sterilize by autoclave (6.4.1).

6.3.8 Tryptone sodium chloride solution (TSCS)

Tryptone, pancreatic digest of casein	1 g
NaCl	8,5 g
Water	1 000 ml

6.4 Laboratory apparatus and glassware

The usual laboratory apparatus and, in particular, the following shall be used.

6.4.1 Autoclave, capable of sterilizing at 121 °C ± 2 °C and 103 kPa ± 5 kPa.

6.4.2 Incubator, capable of being controlled at either 36 °C ± 1 °C or 37 °C ± 1 °C. An incubator at 37 °C ± 1 °C may be used if an incubator at 36 °C ± 1 °C is not available.

6.4.3 pH meter, having an accuracy ±0,1 in calibration on pH units at 25 °C.

6.4.4 Stopwatch.

6.4.5 Container, test tubes or flasks of suitable capacity.

6.4.6 Pipettes, having the most suitable volume for each use, with a tip made of glass or plastic, and with a tolerance of 0,5 % or less.

6.4.7 Petri dishes, that have been sterilized, made of glass or plastic, in diameter sizes of 90 mm to 100 mm.

6.4.8 Luminescence photometer, with ATP kit for water test**,** capable of measuring ATP of 10^{-12} mol/l to 10^{-7} mol/l at 300 nm to 650 nm with a luminescence-measuring reagent.

6.4.9 Vials, 250 ml plastic bottles, with screw openings, polytetrafluoroethylene or silicone packing and caps made of polypropylene, polycarbonate or another suitable material.

6.4.10 Glass beads, with a diameter of 3 mm to 4 mm.

6.4.11 Freezers, one adjustable to a temperature below –70 °C and another to a temperature below –20 °C.

6.4.12 Homogenizer, capable of speeds of six blows per second to eight blows per second, with the corresponding disposable containers.

6.4.13 Disposable plastic bags, sterile bags suitable for containing food products, to be used for one of the shaking methods of the specimens.

6.4.14 Sterilized stirring rod, with a 6 mm (±2 mm) diameter and a 300 mm (±50 mm) length.