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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.

6.5 Preparation of bacterial test suspension

6.5.1 Storage of strains

6.5.1.1 General

The strains shall be stored in accordance with the supplier's recommendations. The experiment instruments should be sterilized by the autoclave (6.4.1) for a minimum of 15 min.

6.5.1.2 Preparation method

Obtain a sample of the freeze-dried bacterial strain in accordance with Annex A, following the recommendations supplied with the culture and resuspend it in 5 ml of TSB (see 6.3.4). Obtain a sample of the suspension and isolate it in a plastic bottle (6.4.10) containing LB (see 6.3.5). Incubate the cultures for 18 h to 24 h at 37 °C ± 2 °C.

After incubation, use the culture isolated in the Petri dish to verify the purity of the strain.

After verification, prepare the stock cultures to check the density of *E. coli* of the bacterial test suspension.

Sample 0,7 ml of the broth culture and spread it over the surface of the Petri dish containing the TSA. Incubate the culture on plates for 18 h to 24 h at 37 °C ± 2 °C.

Add 10 ml of cryoprotective solution (see 6.3.6) to the surface of the TSA plate culture and resuspend the cells in the solution using a sterile glass spreader. Sample the suspended cells from the surface of the agar, dilute them in 100 ml of cryoprotective solution and incubate for 30 min at 20 °C.

Using a pipette (6.4.6), sample 1 ml of the suspension and transfer it to a cryogenic vial (6.4.9) containing the beads (6.4.10). Shake the vial in order to spread the suspended cells around the beads.

— Where a cryoprotective solution containing dimethylsulfoxide is used, do not let it stand longer than 1 min at ambient temperature.

— Where a cryoprotective solution containing glycerol is used, let it stand for 30 min at 20 °C.

— Withdraw the excess cryoprotective solution with a sterile pipette. Place the cryogenic vials in a freezer (6.4.11) set at –70 °C or lower.

Prepare 10^{-6} and 10^{-7} dilutions of the suspension using the serial dilution method. Take a 1,0 ml sample of each dilution and transfer it to separate Petri dishes. Add 12 ml to 15 ml of nutritive solution, cooled down to 45 °C ± 1 °C. Incubate for 18 h to 24 h under the conditions specified for the strain. Enumerate the plate cultures and confirm that the cell density of the suspension is 1×10^5 CFU/ml to 5×10^8 CFU/ml using TSCS (see 6.3.8).

The other preparation details of initial bacteria stock culture are described in EN 1040.[8]

6.5.2 Working culture of test bacteria

In order to prepare the working culture of strains, subculture from the stock culture by inoculation into LB broth and incubates. After 18 h to 24 h, prepare a second subculture from the first subculture in the same way and incubate for 18 h to 24 h. From this second subculture, a third subculture may be produced in the same way.

NOTE The second and/or third subculture are the working culture(s).

If it is not possible to prepare the second subculture on the same day, a 48 h subculture may be used for subsequent sub-culturing, provided that the subculture has been kept in the incubator during a 48 h period. In these circumstances, prepare a further 24 h subculture after proceeding. Do not take a fourth subculture.

6.5.3 Bacterial test suspension

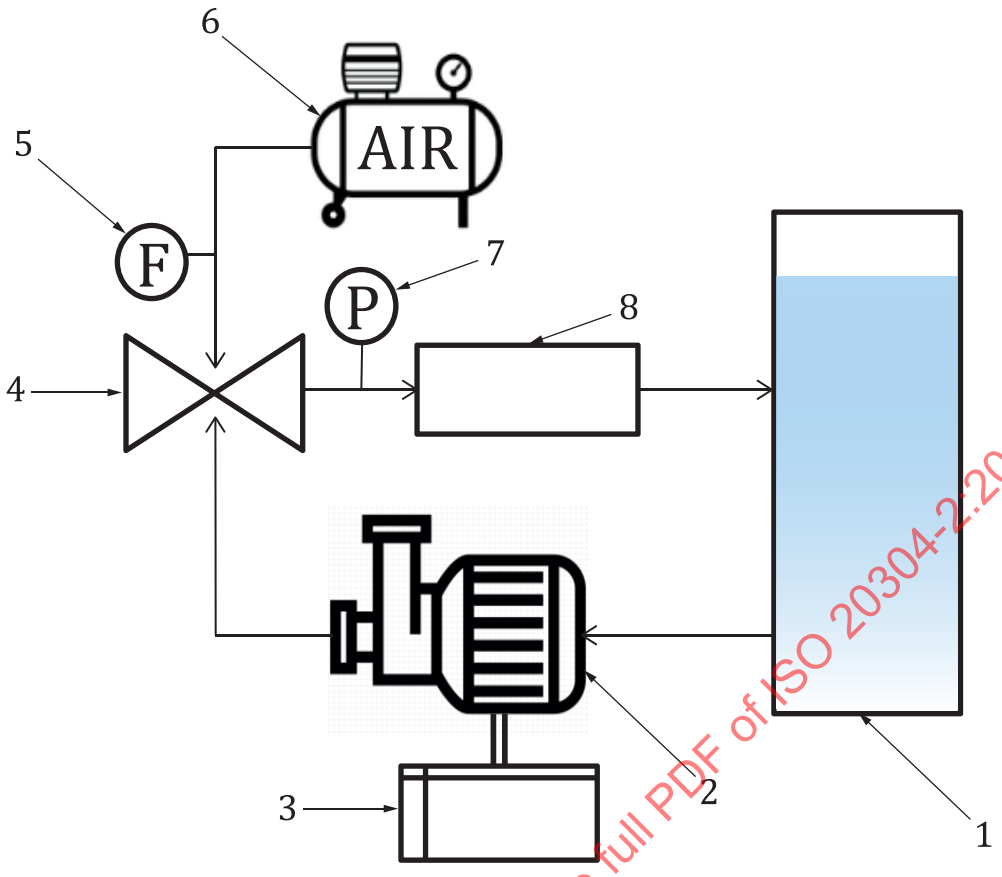
Take the working cultures and transfer into the test tank and adjust the final volume of the bacterial test suspension to 50 l, after it has been diluted with water (see 6.3.2) at room temperature and stir with a stirring rod for 30 s.

Prepare 10^{-1} and 10^{-2} dilutions of the suspension using the serial dilution method. Take a 1,0 ml sample of each dilution and transfer it to separate Petri dishes. Add 12 ml to 15 ml of nutritive solution, cooled down to 45 °C ± 1 °C. Incubate for 18 h to 24 h under the conditions specified for the strain. Enumerate the plate cultures and confirm that the suspension contains more than 1×10^5 CFU/ml. Measure RLU of the sample using luminescence photometer (6.4.8). The principle of RLU shall be as defined in Annex C.

Adjust the final concentration of *E. coli*, which is more than 1×10^5 CFU/ml using luminescence photometer (6.4.8) and working cultured suspension.

6.6 Procedure

The capacity of the pump can be appropriately fixed to circulate the bacterial test suspension within the test facility (see Figure 1). Test fine bubble generating unit shall be operated to produce fine bubbles (see Figure 1). Bacterial test suspension shall be moved to the test tank within 1 h in accordance with Annex B, B.3. The produced fine bubble-enhanced water shall be contacted the bacterial test suspension during the operation of the test facility.



Key

1	test tank	5	air flow meter
2	pump	6	gas
3	control panel	7	pressure gauge
4	gas injector	8	fine bubble generating unit

NOTE The size of pipes and test tank is an example and can be adjustable.

Figure 1 — Experimental set-up for disinfection efficiency test of fine bubbles generated into the water phase

6.6.1 Choice of experimental conditions

The selection of initial contact temperature, initial temperature of the bacterial test suspension, contact time (i.e. operation time) and interfering substances shall be set as follows:

a) initial contact temperature and initial temperature of the bacterial test suspension: approximately 15 °C to 24 °C ± 1 °C;

b) maximum contact temperature: if the contact temperature in the test tank is over 40 °C ± 1 °C, stop the test facility immediately and take samples. The last recorded contact time at the final sampling shall be the last contact time.

c) contact time [operation time of the test facility (see Table 1)] to be tested is 1 min ±30 s; 10 min ±30 s and 30 min ±30 s;

d) stains: *E. coli* (see Table A.1).

Table 1 gives test levels of fine bubbles generated by hydrodynamic cavitation (experiment air temperature range is approximately 15 °C to 20 °C ± 3; water temperature of the experiment liquid medium is 15 °C to 40 °C).

Table 1 — Test design and description of each test level

	Test Level	Description (measurement solution)
Bacteria test suspension with fine bubbles	Test Level I	Bacteria test solution generated by a fine bubble cavitation unit
Control suspension	Test Level II (control)	Bacteria test solution produced without using fine bubble generation (control solution) (i.e. the liquid circulation in the pipe generated turbulence can cause a potential decrease of the bacteria count)

6.6.2 Test procedure for antibacterial activity

In this document, the test facility shall be operated for the two main tests which are Level II (control test; bacterial test suspension + without fine bubble generation) and Level I (bacterial test suspension + fine bubble generation) test. After each test, three replicated sampling for the test solution shall be conducted at each contact time and at each bacteria strain for the analysis (see Table 2). After the test trials for Level II, adjustment of the tests for Level I should be carried out. The proper washing and disinfection procedure for the test facility should be carried out between the tests for each test bacteria.

For each test, measure the value of RLU of the initial bacterial test suspension (N) as specified in 6.5.3, and the value of RLU after each test (N_a), respectively.

Table 2 shows the table frame of the test results on the disinfection efficiencies (D) of fine bubble test levels estimated by each test bacterial strain's disinfection test (test bacteria: *E. coli* ATCC 11775).

Table 2 — Table frame of test results

Test case	Indices	Contact time			Bacterial test suspension
		1 min ±30 s	10 min ±30 s	30 min ±30 s	
Test Level I	Initial ATP concentration index (RLU)	N_a	N_a	N_a	N
	Reduction in viability (rate)	R	R	R	
	Disinfection efficiency index	D	D	D	
Test Level II (control)	Reduction in viability (rate)	N_a	N_a	N_a	N
	Disinfection efficiency (index)	R_c	R_c	R_c	

N = amount of ATP concentration index of the bacterial test suspension
N_a = amount of ATP concentration index of each test solution
R = reduction in viability of each test solution
R_c = reduction in viability of the control test
D = disinfection efficiency of the test fine bubbles

6.7 Calculation and expression of results

For each test sample, the amount of ATP in the bacterial test suspension (N) and after the test procedure for antibactericidal activity of the fine bubbles (N_a) are recorded. The antibactericidal activity of the test facility is expressed as reduction in viability (R) as shown in Formula (1):

$$R = 1\text{-}(N_a / N) \quad (1)$$

For each test case of level I and Level II (control), reduction in viability (R) value is calculated. The disinfection efficiency of the test fine bubble is expressed as shown in Formula (2):

$$D = R/R_c \quad (2)$$

— Where D is a disinfection efficiency index of the test fine bubbles; R is the reduction in viability of test fine bubble mixture and R_c is the reduction in viability of the control test. If the test value of D is bigger than 1, the test fine bubble system has disinfection function.

Annex A
(normative)

Strain numbers

A.1 General

The bacteria to be used in the test shall be identical to those listed in Table A.1. The strains used in the test shall be stated in the test report (see Annex D) together with their supply sources.

A.2 List of bacteria

Table A.1 — Bacteria for testing

Bacteria type	Available strains	Biosafety level
Escherichia coli	ATCC11229, ATCC9637, ATCC 8739, ATCC13706, ATCC 23631, ATCC700891, ATCC700609, and ATCC700078	BSL 1
	ATCC11775, CCM5172, BCRC10675, CCUG24, CCUG29300, CIP54.8, CN4382, DSM30083, IAM12119, JCM1649, LMG2092, NBRC102203, NCCB54008, NCDO1989, NCIMB11943, NCTC9001	BSL 2

NOTE Other bacteria can be used after appropriate validation.

Annex B
(normative)

Shaking method

B.1 General

There are three methods to shake-out the bacteria from specimen, which are shaking by vortex mixer, shaking by hand and shaking by a homogenizer (6.4.12).

B.2 Shaking by mixer

Press the bottom portion of the test tubes or bottles on the plate or rubber holder of the vortex mixer and mix for 5 s × 5 cycles.

B.3 Shaking by hand

Take the test bottle or container by hand and shake in an arc of approximately 30 cm for 30 s.

B.4 Shaking by homogenizer

Place the designated disposable bag (6.4.13) in the homogenizer (6.4.12) and run the machine for 1 min on each face of the bag.

Annex C
(normative)

Luminescence photometers and RLU

C.1 General

This annex provides general principles of luminescence photometers and RLU.

C.2 Principle of luminescence photometers

Luminescence photometers normally use a photomultiplier tube (PMT); photons striking a photocathode at the entrance window of a PMT produce electrons, which are then accelerated by a high-voltage field and multiplied in number within a chain of dynodes by the process of secondary emission, and reach the anode connected to an output processing circuit. Then, this is translated to an output signal which can be a pulse (if the PMT works in photon counting mode) or an analog current if the PMT works in analog mode (also known as current mode).

The output is affected by the PMT mode, the voltage applied, the efficiency of the PMT and other parameters. The output differs from different instruments. Therefore, the basic information of the luminescence photometers used should be specified in the test results.

C.3 RLU

Relative light units (RLU) are the units used for luminescence measurements. RLU do not have any physical meaning and the absolute results differ from each instrument. Sensitivity of RLU varies from different measurement products and is used as a relative index to measure the disinfection efficiency of the tested fine bubbles. Therefore, this unit should be used as an efficiency unit. In the present study, it is used to estimate the disinfection efficiencies of the test fine bubbles.

Annex D
(informative)

Test report of interlaboratory tests for disinfection efficiency of fine bubbles

D.1 Test report

The test report shall include, at least, the following information:

a) a reference to this document, i.e. ISO 20304-2:2024;

b) the identification of the laboratory;

c) the identification and capacity of test fine bubble cavitation unit;

— name and number of the product model;

— manufacturer;

— capacity, mean size and number concentration of the test fine bubbles generated;

— identification of bubble gas; amount and general operating condition;

— main application purpose;

d) the test method;

— full details of the test facility;

— basic information of luminescence photometer used;

e) the experimental conditions;

— experiment time;

— test air temperatures;

— model of luminescence photometer used;

— identification of bacterial strains used;

f) the test results (see Table 2);

— antibacterial activity and disinfection efficiency of the test fine bubbles;

g) other comments;

h) the locality, date and identified signature.

D.2 Interlaboratory tests and the results

Three interlaboratory test results are shown in the followings. The same test facility setting was used for the interlaboratory tests.

D.2.1 Fine bubbles generator system and test facility (see Figure 1)

The test fine bubble generation system is a domestic fine bubble shower tap nozzle. The system adopts the normal air gas, PVC pipe line (15 mm in diameter) and PMMA (poly methyl methacrylate) test tank. [500 mm (width) × 500 mm (length) × 700 mm (height)]. Sampling point from the test tank is 50 mm below the surface of the bacterial test suspension. The capacity of the pump is 220 V, 60 Hz, 0,4 KW. The outlet flow amount is 11 (±1,0) LPM, the outlet pressure is 2 bars and the air ratio injected is 0,2 %.

D.2.2 Test report-1

a) Identification of laboratory: IrehEnvit Laboratory, Pajusi, Gyeonggido, Korea.

b) Identification and capacity of test fine bubble cavitation unit:

   1) Name and number of the product model: domestic tap-water fine bubble nozzle.

   2) Manufacturer.

   3) Capacity, mean size and number concentration of the test fine bubbles generated:

      — Measurement instrument.

      — Software.

      — Light source used: 488 nm (blue).

      — Used water: normal tap-water.

      — Average bubble density: control: 8,87 × 10^6; test: 3,92 × 10^9/ml.

      — Average bubble diameter: 247 nm.

   4) Identification of bubble gas; amount and general operating condition: general air; injected air ratio: 0,2 %.

   5) Main application purpose; domestic use for tap-water (disinfection of shower horse).

c) Experimental conditions:

   1) Experiment time; approximately 10:00 pm to 11:30 pm, 20 August 2021.

   2) Test air temperature; 23 °C.

   3) Luminescence photometer used.

   4) Identification of bacterial strains used; *E. Coli* ATCC11775.

d) Test results (see Table D.1):

   1) Antibacterial activity and disinfection efficiency of the test fine bubbles.

Table D.1 — Test results at IrehEnvit laboratory

Test case	Indices	Contact time			Bacterial test suspension
		1 min ±30 s	10 min ±30 s	30 min ±30 s	
Test Level I	Initial ATP concentration index (RLU)	N_a: 9,541(±194,6)	N_a: 2,266(±32,5)	N_a: 1,708(±100,7)	*N:* 10,001(±256,6)
	Reduction in viability (rate)	*R*: 0,046	*R*: 0,773	*R*: 0,829	
	Disinfection efficiency index	*D*: 0,726	*D*: 7,703	*D*: 2,579	
Test Level II (control)	Initial ATP concentration index (RLU)	N_a: 7,282(±189,0)	N_a: 6,993,8(±87,8)	N_a: 5,274(±244,9)	*N:* 7,774(±80,1)
	Reduction in viability (rate)	R_c: 0,063	R_c: 0,100	R_c: 0,322	

N = amount of ATP concentration index of the bacterial test suspension

N_a = amount of ATP concentration index of each test solution

R = reduction in viability of each test solution

R_c = reduction in viability of the control test

D = disinfection efficiency of the test fine bubbles.

e) Other comments; none.

f) Date, locality and identified signature; August 20 2021; Paju, Gyeounggido, Rep. of KOREA; Mr. Hun Young LEE.

D.2.3 Test report-2

a) Identification of laboratory; Department of Environmental Engineering, Inha University, Incheon, Korea.

b) Identification and capacity of test fine bubble cavitation unit:

1) Name and number of the product model: domestic tap-water fine bubble nozzle.

2) Manufacturer.

3) Capacity, mean size and number concentration of the test fine bubbles generated:

— Measurement instrument.

— Software.

— Light source used: 488 nm (blue).

— Used water: normal tap-water.

— Average bubble density: control: 8,87 × 10^6; test: 3,92 × 10^9/ml.

— Average bubble diameter: 247 nm.

4) Identification of bubble gas; amount and general operating condition: general air; injected air ratio: 0,2 %.

5) Main application purpose; domestic use for tap-water (disinfection of shower horse).

c) Experimental conditions:

1) Experiment time; approximately 13:30 pm to 15:00 pm, 17 September 2021.

2) Test air temperature; 20 °C.

3) Luminescence photometer used.

4) Identification of bacterial strains used; *E. Coli* ATCC11775.

d) Test results (see Table D.2):

1) Antibacterial activity and disinfection efficiency of the test fine bubbles.

Table D.2 — Test results at Inha university

Test case	Indices	Contact time			Bacterial test suspension
		1 min ±30 s	10 min ±30 s	30 min ±30 s	
Test Level I	Initial ATP concentration index (RLU)	N_a: 10 579,8(±542,5)	N_a: 4 362,6(±231,3)	N_a: 2 186,8(±199,1)	*N:* 12 222,2(±102,8)
	Reduction in viability (rate)	*R*: 0,134	*R*: 0,643	*R*: 0,821	
	Disinfection efficiency index	*D*: 0,703	*D*: 2,202	*D*: 2,128	
Test Level II (control)	Initial ATP concentration index (RLU)	N_a: 7,282(±189,0)	N_a: 6,993,8(±87,8)	N_a: 5,274(±244,9)	*N:* 7,774(±80,1)
	Reduction in viability (rate)	R_c: 0,191	R_c: 0,292	R_c: 0,386	

N = amount of ATP concentration index of the bacterial test suspension

N_a = amount of ATP concentration index of each test solution

R = reduction in viability of each test solution

R_c = reduction in viability of the control test

D = disinfection efficiency of the test fine bubbles

e) Other comments; none.

f) Date, locality and identified signature; 17 August 2021; Incheon, Rep. of KOREA; Mr. Hyungjun KIM.

D.2.4 Test report-3

a) Identification of laboratory; Department of Civil Engineering Seoul National University, Gwanak-ro 1, Gwanak-gu, Seoul, Korea.

b) Identification and capacity of test fine bubble cavitation unit:

1) Name and number of the product model: domestic tap-water fine bubble nozzle.

2) Manufacturer.

3) Capacity, mean size and number concentration of the test fine bubbles generated:

— Measurement instrument.