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Fine bubble technology — Agricultural applications —

Part 2: Test method for evaluating the promotion of the germination of barley seeds

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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 23016 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

Considering the increasing use of fine bubble technology in agriculture, this document has been developed to establish standards in this area, with particular focus on promoting the germination and growth of barley seeds.

The use of fine bubble technology in agriculture has been confirmed to benefit various types of agricultural products and has attracted the interest of various countries. Application of the technology to leafy vegetables in agriculture is already well-established, and this is being expanded to seed germination and growth as well. Worldwide as well, standardization of fine bubble technology in the field of agriculture is not only being spotlighted but is being conducted in practice at a rapid pace. The technology is expected to blossom rapidly.

Fine bubble technology has been applied successfully not only in agriculture but also in the fields of environmental science, food, marine products, medicine, etc. Wide-ranging progress in standardizing the technology is being made in these fields. The achievement of standardization in various fields is expected to result in increased worldwide recognition of fine bubble technology in the future.

Fine bubble technology — Agricultural applications —

Part 2: Test method for evaluating the promotion of the germination of barley seeds

1 Scope

This document specifies a method to test the promotion of the germination of barley seeds, using ultrafine bubble (UFB) water produced from an ultrafine bubble water generating system. The performance of the method is assessed by measuring the ratio of barley seed germination.

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 20480-1, *Fine bubble technology — General principles for usage and measurement of fine bubbles — Part 1: Terminology*

ISO 20480-2, *Fine bubble technology — General principles for usage and measurement of fine bubbles — Part 2: Categorization of the attributes of fine bubbles*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 20480-1 and ISO 20480-2 and the following 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
germination
appearance of a sprout of at least 1 mm of length

[SOURCE: ISO 18763:2016, 3.7, modified — "root" has been replaced by "sprout".]

3.2
immersion
act of immersing unprocessed barley seeds in ultrafine bubble water or control water

3.3
ultrafine bubble generating system
UFB generating system
equipment that uses water and air to generate ultrafine bubbles by mechanical action

Note 1 to entry: Ultrafine bubbles (UFB) are bubbles with a diameter of less than 1 µm. See ISO 20480-1.

3.4
ultrafine bubble water
UFB water
water that includes UFB

Note 1 to entry: UFB are defined in ISO 20480-1.

3.5
control water
water for use as a control sample for the effects of UFB

3.6
raw water
distilled water supplied as a raw material for both *UFB water* (3.4) and *control water* (3.5)

3.7
test water
either *UFB water* (3.4) or *control water* (3.5)

3.8
UFB section
test beaker containing *UFB water* (3.4) for use in germination tests of barley seeds in UFB water

3.9
control section
test beaker containing *control water* (3.5) for use in germination tests of barley seeds in control water

3.10
growth period
period from sowing of seeds through the final germination observation

3.11
germination ratio
G
ratio of the seeds observed during inspection to have germinated to the total number of seeds provided for the test

3.12
UFB enabled efficiency
P value
quantity measuring the efficiency of UFB for its enhancement in the germinating period

Note 1 to entry: *P* is defined by Formula (1).

4 General testing principle

4.1 General overview of the test system

UFB water shall be generated through the supply of raw water to the UFB generating system. The UFB water shall be supplied to the UFB section to promote the germination of the barley seeds that have been supplied to the UFB section. In addition, raw water for control purposes that does not contain UFB shall be supplied directly to the control section. Figure 1 shows the constitutive principle of the growth promotion performance test.

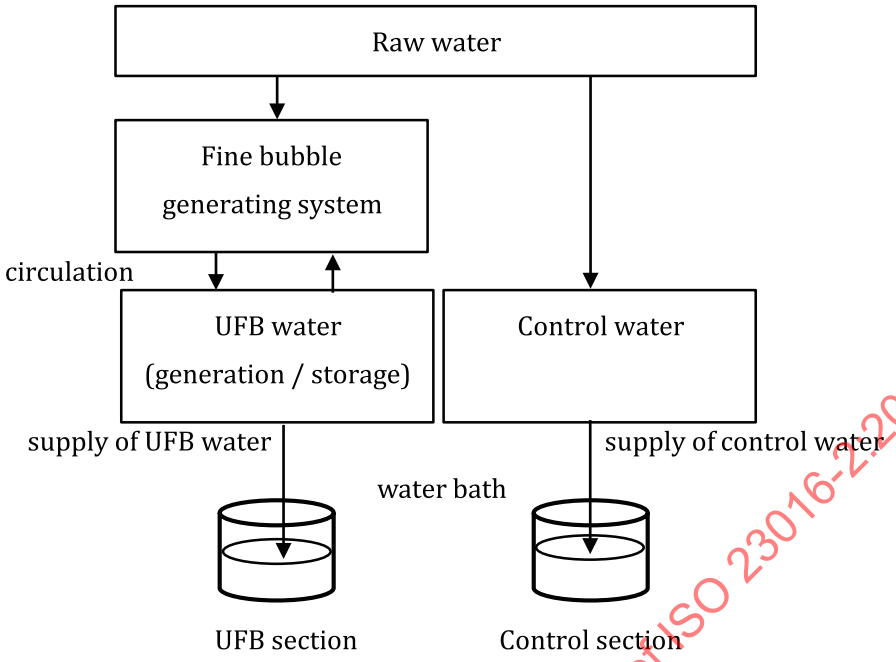


Figure 1 — General view of growth promotion performance test system

4.2 Assessment of test results

The germination ratio G is obtained by calculating the number of germinated seeds to the number of all seeds tested for each inspection.

The development of the germination ratio is assumed to be expressed by a specified S-curve regression function[2],[3] and a parameter T_{50} related to the delay in the germination is to be inferred by the process as described below for each section. The relative difference of the parameter for UFB section to control section is the measure for the performance of the fine bubble enhanced performance, defined as efficiency, P, to be reported.

Efficiency, P, is given by Formula (1):

$$P = \left(T_{50}\left(\text{control}\right) - T_{50}\left(\text{UFB}\right)\right) / T_{50}\left(\text{control}\right) \tag{1}$$

where

P is the efficiency;

T_{50} (control) is the T_{50} of seeds in the control water;

T_{50} (UFB) is the T_{50} of seeds in the UFB water.

The S–curve regression function is given by Formula (2) by data fitting using the least square method over all inspections and samples for each section.

$$G(t_i) = f(t_i, B, G_{\max}, T_{50}) = G_{\max} / \left[1 + \exp\left(B\left(\log(t_i) - \log(T_{50})\right)\right)\right] \tag{2}$$

where

G_{max} is the maximum germination ratio of UFB section;

t_i is the time for each inspection; it is recommended to use $t_{0,1}$ for the time zero instead of t_0, in order to avoid the calculation of log 0 for the smooth data analysis by software;

T_{50} is the time when the inferred germination ratio is 50 % of G_{max};

$G(t_i)$ is the observed germination ratio for each inspection;

B is the slope at the time T_{50}.

An example of the assessment is shown in Annex A.

NOTE Once a seed quality is fixed, the P value evaluated right by T_{50} represents UFB-enabled performance of UFB water and UFB generating system.

5 Test subjects

The items subject to the test specified in this document shall be UFB water stored in bottles or other containers for preservation and transport, and the UFB generating system used to generate UFB water. UFB water shall be generated by supplying raw water to the UFB generating system. Distilled water with a quality of A2[1)] or greater shall be used as raw water.

The size, quantity and concentration of UFB in UFB water shall be measured or given.

The UFB generating system shall be capable of supplying the aforementioned UFB water in the same manner to containers that are suitable for preservation and transport.

6 Apparatus and test equipment

6.1 Seeds for germination test

Barley seeds shall be used as the seeds for the germination test. Barley seeds can be obtained from various sources. The seeds shall not be rinsed in water for use; the test shall be initiated using the dry seeds as is.

6.2 UFB generating system and UFB water

UFB water shall be generated by supplying raw water to the UFB generating system. For the purpose of this document, the UFB generating system refers to all equipment that can be connected together and operated: the UFB generating system, the control water supply system and the UFB water supply system. Prior to the use of UFB water, the UFB water shall be left stationary until white turbidity caused by micro bubbles generated at the same time as UFB turns clear as these micro bubbles float in the water and disappear.

However, the maximum storage time until UFB are used for the test shall be approximately 48 h in generally used bottles.

6.3 Measurement and observation equipment

The following equipment should be prepared:

a) pH meter;

b) electric conductivity (EC) meter;

1) Electrical conductivity 0,1 mS/S (25°C), total organic carbon (TOC) 0,5 mgC/l or less, zinc 0,5 µgZn/l or less, silica 50 µgSiO_2/l or less, chloride ions µgCl^-/l, sulfide ions µgSO_4^{2-}/l.

c) DO (dissolved oxygen concentration) meter;

d) thermometer;

e) clock;

f) magnifying viewer.

6.4 Beakers

Four glass beakers with a volume of 2 l or greater shall be prepared. A volume of 1,5 l (1 500 ml) of UFB water and control water shall be poured into these beakers. Before use, they shall be washed with the distilled water to be used for UFB generation. Three times washing is enough. The four beakers shall be allocated as follows (see Figure 3):

a) for control water (seeds immersed);

b) for UFB water (seeds immersed);

c) for control water (replacement);

d) for UFB water (replacement).

6.5 Constant-temperature water tank

The four beakers prepared in 6.4 shall be placed in a constant-temperature water tank capable of controlling the temperature of a large quantity of water (tap water may be used) in the tank to maintain the temperature of the test water in the beakers at a stable temperature between 20 °C and 30 °C.

6.6 Plastic bags

Six net-like plastic bags to contain the specified number of seeds for the test shall be prepared. The bags shall prevent the seeds from moving or spilling out, etc. The holes in the net shall be large enough to allow the test water to pass through easily but small enough to prevent the seeds from passing through. Mesh size between 14 and 40 is recommended as a common type.

6.7 Test tray and filter paper

Two trays of a size sufficient to accommodate filter paper measuring approximately 185 mm in diameter shall be prepared.

Filter paper of 18 cm or more in diameter on which seeds are arranged to check germination.

6.8 Limit sample for inspection

The limit sample is shown in Annex B.

The inspection shall be carried out, using a magnifying viewer and the limit sample, by personnel with the capacity to clearly distinguish whether germination has been achieved.

6.9 Maintenance of measurement devices and equipment

6.9.1 Maintenance

The performance of the measurement devices and equipment shall be checked and maintained regularly.

6.9.2 Calibration

Each measurement device shall be calibrated regularly after checking the control record for that device, in order to maintain the reliability of measurement data.

7 Test environment

7.1 Temperature conditions

The temperature of the test water in the beakers shall be maintained between 20 °C and 30 °C.

The air temperature in the testing room shall be maintained constantly at normal temperature.

Place a plastic cover on the top of the beaker to prevent the effect of wind, evaporation of moisture, intrusion of dust etc.

7.2 Environmental parameters to be measured

a) pH (test water).

b) Electric conductivity (test water).

c) Dissolved oxygen (test water).

8 Test procedure

8.1 Test period and interval between inspections

a) The test period shall be established based on information from the source from which the seeds were acquired. If this information cannot be obtained, a preliminary test shall be performed to establish the test period.

b) The inspection intervals shall be established so that inspections are performed at least four times during the test period.

8.2 Seed selection and packing into bags

a) The seeds for the test shall be selected from a quantity of seeds that is large enough to enable 300 seeds to be selected. First, the small seeds and foreign matter shall be removed. Then the seeds shall be selected using the limit sample (see Annex B), based on the following criteria:

   1) seeds are large and of a uniform size;

   2) no brown or black discoloration or the like, and seeds are a uniform colour;

   3) no damage on the surface;

   4) non-germinated seeds.

b) Use 300 seeds selected based on the criteria in a). Divide the 300 seeds into two groups of 150 seeds each. Place the first group in the UFB section and place the second group in the control section. Divide the seeds in each group into three groups of 50 seeds each and insert them into net-like plastic bags (see Figure 2). For the group of seeds to be immersed in the UFB water, place each group of 50 seeds into a bag so there are three bags. Similarly, for the group of seeds to be immersed in the control water, place each group of 50 seeds into a bag so there are three bags.

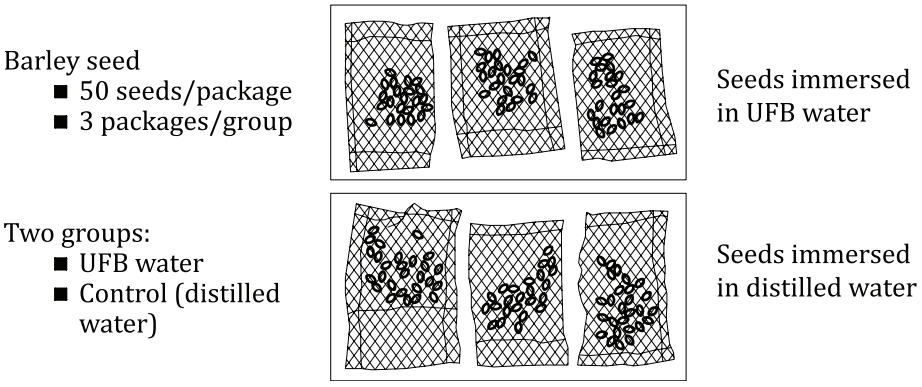


Figure 2 — Seeds in plastic bags —Use of UFB water and control water

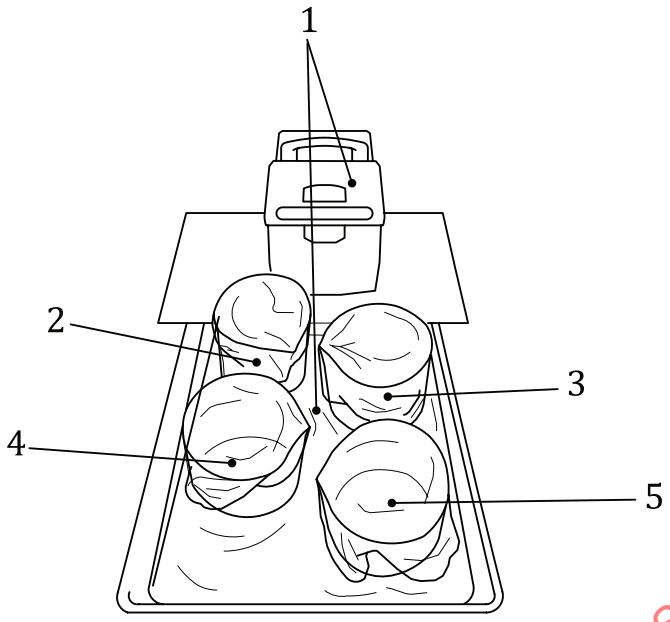
8.3 Use of UFB water and control water

Prepare four beakers containing UFB water (UFB section) and control water (control section). Two of the beakers shall be used for the test and shall contain test water, and two groups of 150 seeds each shall be provided for the test. The remaining two beakers shall contain replacement test water. The four beakers shall be placed in a constant-temperature water tank containing a large quantity of water maintained at a temperature between 20 °C and 30 °C in order to maintain the temperature of the test water. At least 10 ml of test water for each seed shall be prepared, which represents a total volume of 1,5 l of water for 150 seeds (50 seeds × 3 bags) (see Figure 3).

a) Immerse the three bags of 50 seeds each into each of the types of test water and protect them from the light.

b) Once each half-day, remove the bags containing the seeds from the test water and replace each of the types of test water with fresh test water. Make sure the process of replacing the test water takes no more than approximately 2 min to ensure that the removed seeds do not dry out.

A volume of 20 ml of test water per seed may be provided. In such cases, it shall only be necessary to replace the test water once per day (3,0 l of water for 150 seeds). However, the test shall be performed after confirming that the same results as in a) above are achieved.



Key

1 water bath and temperature controller, temperature 25 °C
2 spare distilled water
3 control (distilled water)
4 UFB water
5 spare UFB water

Figure 3 — Beakers whose temperature is controlled in the constant-temperature water tank (UFB water and control water)

8.4 Tray preparation and seed sowing

a) Place a filter paper measuring approximately 185 mm in diameter on the tray and moisten it with enough test water to ensure that the seeds will not dry out.

b) Remove the plastic bags containing the seeds and use tweezers to line the seeds up in the bags so they can be observed from above (see Figure 4). Arrange the 50 seeds in each bag so there are 5 orderly rows of 10 seeds each.

c) Take photographs of each inspection.

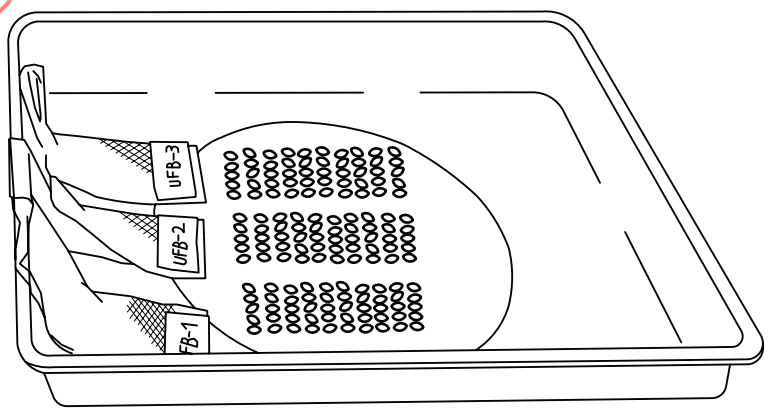


Figure 4 — Arrangement of seeds

8.5 Inspection and recording

a) Count the seeds that have germinated, using a magnifying viewer if necessary. Assess whether or not the seeds have germinated in accordance with the limit sample (Figure B.1).

b) After quickly recording the number of seeds that have germinated, return all the seeds to the plastic bags and immerse them in the test water again.

c) Record the number of germinated seeds at appropriate intervals ranging from 6 h to 12 h, and conclude the test when the specified test period has ended.

d) Calculate the germination ratio for each bag at each measurement time and express the result as a percentage value (%). Calculate the ratio for germinated seeds/50 (%) and determine the mean value and standard deviation for the three plastic bags.

NOTE With good quality barley seeds, germination will occur in one to two days. With poor quality seeds, germination will take several days to approximately one week.

9 Test report

The test report shall contain the following items:

a) test institution and test date;

b) type of seeds: Naked barley [*Hordeum vulgare* L.];

c) names of test subjects; for example, “UFB water A, UFB generating system B”;

d) when testing a UFB generating system, operational parameters for obtaining UFB water that can be adjusted by the user;

e) number of test seeds: 300 seeds per test;

f) environmental test conditions i.e. water temperature, room temperature, pH, water electrical conductivity, DO, UFB concentration value and UFB mean/mode value (values of the last two items can be based on actual measurement or from the equipment supplier’s information);

g) test parameters i.e. test period, inspection interval, number of inspections;

h) *P* value;

i) any deviations from the procedure or any unusual features observed.

Annex A
(informative)

Example of test result at testing laboratory

A.1 Test procedure

The test procedure conducted at the testing laboratory is as specified in this document.

A.2 Test result

Figure A.1 shows the development of the germination ratio with respect to each inspection time over four inspections and illustrates the acceleration in germination enabled by application of UFB water.

Figure A.1 shows the results of optimization of the S-curve regression function. The parameters T_{50} at the time up to 50 % of G_{max} are obtained as follows:

Control: 73,15 h ± 1,66 h

UFB: 39,11 h ± 0,87 h

P = (46 ± 2) %