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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

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](http://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](http://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](http://www.iso.org/iso/foreword.html).

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](http://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.

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# 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*

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## 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.



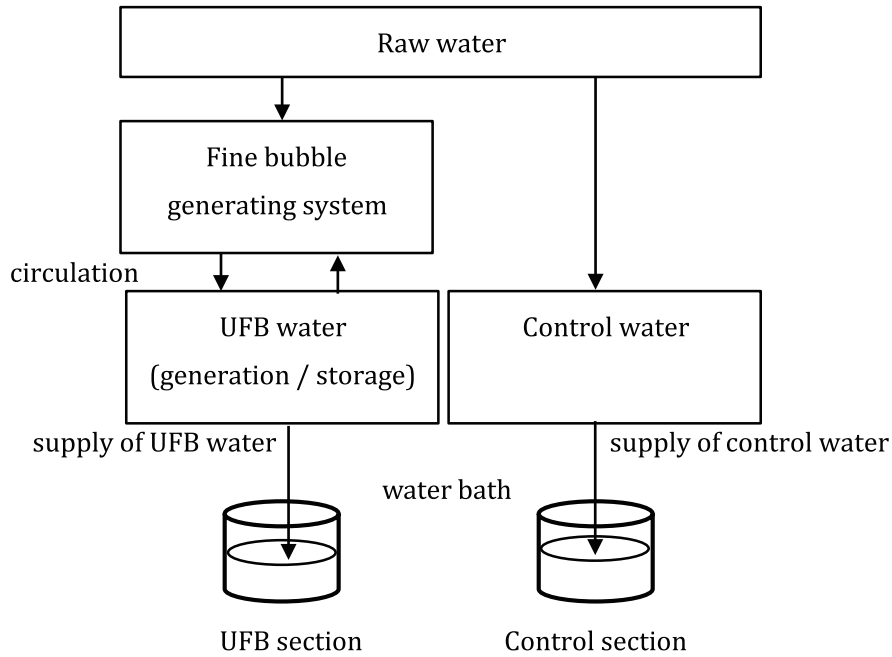


Figure 1 — General view of growth promotion performance test system

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):

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

where

- $P$  is the efficiency;
- $T_{50}(\text{control})$  is the  $T_{50}$  of seeds in the control water;
- $T_{50}(\text{UFB})$  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.

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

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<sup>1)</sup> 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

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### 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;

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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<sub>2</sub>/l or less, chloride ions µgCl<sup>-</sup>/l, sulfide ions µgSO<sub>4</sub><sup>2-</sup>/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.