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Fine bubble technology — Agricultural applications —

Part 4: Test method for evaluating the number concentration of ultrafine bubbles (UFB) achieving the promotion of barley seed germination

PROOF/ÉPREUVE

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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

Fine bubble (FB) technology is increasingly attracting attention in numerous different fields. In the agriculture and aquaculture fields, ultrafine bubbles (UFBs) have been widely and practically used to accelerate the growth of plants and fish (air or oxygen UFBs). However, a lot of cases reported in recent years are field data, some of which are not supported statistically.

In the light of this situation of scientifically insufficient information on fine bubble technology in agriculture, this document has been developed to establish standards in this area focusing on a method to find an adequate number concentration of UFBs demonstrating the effect for promoting the germination of barley seeds.

A test method for the promotion of the germination of barley seeds has been published as ISO 23016-2 and a guideline of the minimum viable number concentration of UFBs for promoting the germination of barley seeds has been published as ISO/TR 23016-3. Successive accumulation of data, however, revealed that a positive/negative effect on germination appears depending on the variety of barley seed. Therefore, a method to assess the UFB number concentration which assures the promotion of germination irrespective of variety is needed for popularizing this technology.

Fine bubble technology — Agricultural applications —

Part 4: Test method for evaluating the number concentration of ultrafine bubbles (UFB) achieving the promotion of barley seed germination

1 Scope

This document specifies a method to assess the ultrafine bubble (UFB) number concentration in order to find whether the number concentration of UFB generated by users is in the adequate range for promoting the barley seed germination stably irrespective of seed variety conforming to ISO 23016-2 and ISO/TR 23016-3.

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 23016-2:2019, *Fine bubble technology — Agricultural applications — Part 2: Test method for evaluating the promotion of the germination of barley seeds*

ISO/TR 23016-3, *Fine bubble technology — Agricultural applications — Part 3: Guidelines for the minimum viable number concentration of ultrafine bubbles for promoting the germination of barley seeds*

ISO 21255, *Fine bubble technology — Storage and transportation of ultrafine bubble dispersion in water*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 23016-2 and ISO/TR 23016-3 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 https://www.electropedia.org/

4 Requirements for UFB water used

The items subject to the test shall be air UFB water stored in bottles or other containers for preservation and transport in accordance to ISO 21255, 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 according to ISO 23016-2.

The size, quantity and concentration of UFB in UFB water shall be measured. For the generation of air UFB in water, a pressure dissolution system, whose pressure just after the pressurizing pump is around 700 kPa and that at the saturator is around 300 kPa was used. For measurement, a commercial

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.

device using particle tracking analysis (PTA) was used, whose measuring range is from 50 to 1 000 nm, wave length of laser light source is 635 nm, power is 40 mW, mounted camera is black and white CCD and analysis software is NTA 3.1 Build 3.1.46. Setting parameters are given in Annex A. Measuring temperature was at a room temperature around 22 °C.

The UFB generating system shall be capable of supplying the UFB water described in ISO 23016-2.

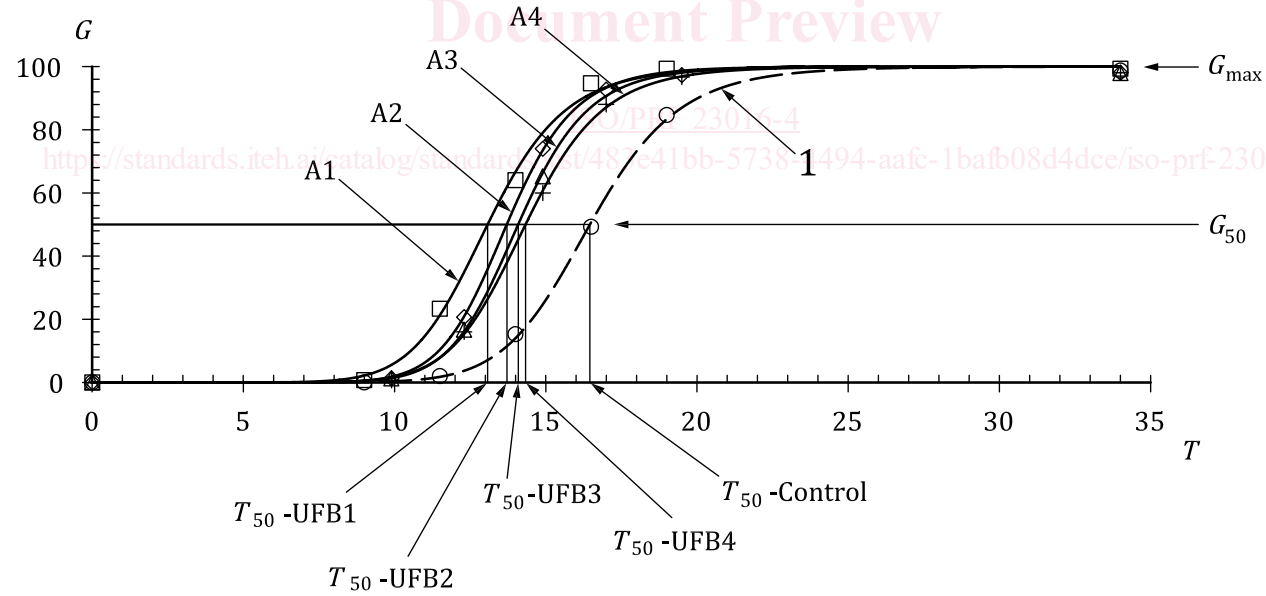
5 Basic characteristics of UFB water relevant to promotion of barley seed germination[1]

Barley seeds of the variety 'Kobinkatagi' are used as a baseline example and they are selected according to the test procedure in ISO 23016-2.

Hollow circles in Figure 1 show the observed germination ratio at each observation time of seeds in control water (distilled water) and its regression curve (S-curve) is drawn in dashed line by using ISO 23016-2:2019, Formula (2). In this case, median germination time, T_{50}-control, was 16,5 h.

Hollow rectangle, solid diamond, hollow triangle and cross symbols in Figure 1 show the observed germination ratios at each observation time of which number concentrations of UFB1, UFB2, UFB3 and UFB4 are $8{,}5\times10^8$/ml, $5{,}0\times10^8$/ml, $3{,}8\times10^8$/ml and $1{,}4\times10^8$/ml and T_{50}-UFB1, T_{50}-UFB2, T_{50}-UFB3 and T_{50}-UFB4 are 13,1 h, 13,7 h, 14,1 h and 14,3 h, respectively. Each of the four regression curves is drawn in solid line in Figure 1.

These experimental observations indicate that the relationship between T_{50}-UFB and T_{50}-control is given as T_{50}-UFB<T_{50}-control which means the improvement in germination speed in the UFB section when number concentration of UFB is in the adequate range for promoting seed germination. Thus, the basic characteristics of UFB water appears in the way that the effect of UFB water on seed germination increases with increasing number concentration of UFB.



Key

○	data at control section
□	data at UFB1 section
◇	data at UFB2 section
△	data at UFB3 section
+	data at UFB4 section
T	measurement time, expressed in hours
G	germination ratio, expressed in per cent

Ai	regression curve of germination ratio of seeds in UFB i water, i = 1 to 4
B	regression curve of germination ration of seeds in control water
G_{max}	point to reach the maximum germination ratio, expressed in per cent
G_{50}	point to reach 50 % of maximum germination ratio, expressed in hours
T_{50}-control	crossing time with G_{50} and the curve of control section, expressed in hours
T_{50}-UFBi	crossing time with G_{50} and the curve of UFBi section, expressed in hours, i = 1 to 4
1	control

Figure 1 — Promotion of UFB on germination of barley seeds in the case that four different UFB number concentrations from UFB1 to UFB4 are in the adequate range for promoting seed germination (Kobinkatagi)

6 Assessment of the adequacy of UFB number concentration[1]

6.1 General

Prepare two UFB waters with different number concentrations, UFB1 and UFB2, and compare T_{50} of each germination process of barley seeds in UFB waters and in control water determined from the S-curve regression function. Here, the number concentration of UFB1 shall be higher than that of UFB2.

As a precondition, it shall be followed that barley seeds of the same quality are used. Being of the same quality is defined as such that the seeds are stored under the same conditions such as storage duration and storage temperature.

In order to assess the adequacy of UFB number concentration for promoting seed germination, the following three cases are considered sufficient.

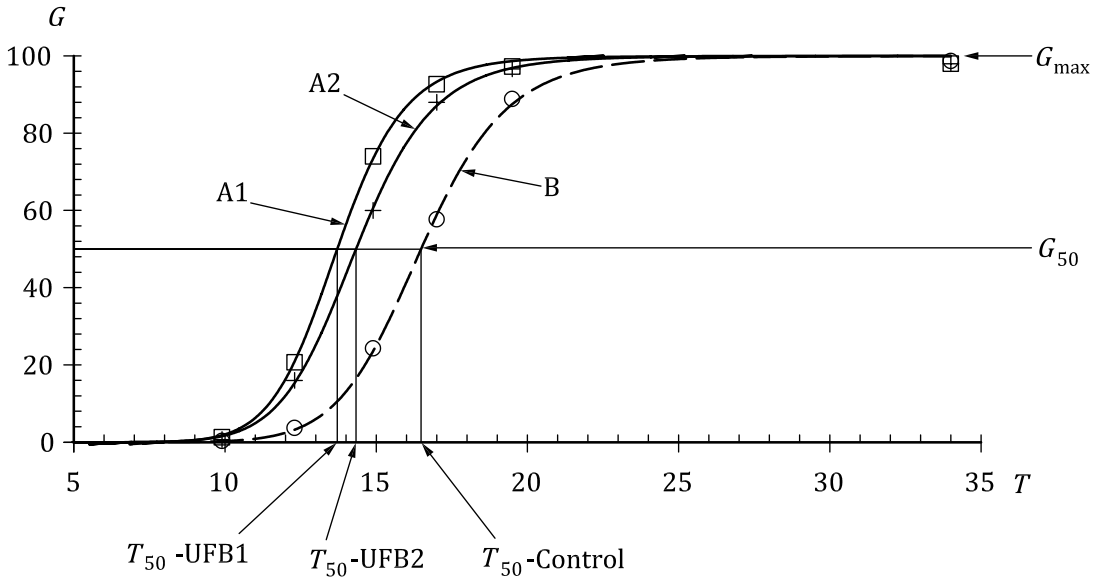
Barley seeds of the variety 'Kobinkatagi' are used in 6.2 and those of the variety 'Yumesakiboshi' are used in 6.3 and 6.4. Adding to this, barley seeds of the variety 'Ichibanboshi' are introduced in 6.3 to indicate the difference in UFB effect between the 'Yumesakiboshi' and 'Ichibanboshi' varieties of which figures are shown in Annex A. All seeds' storage duration and storage temperature are shown in Annex B.

6.2 Case 1: UFB1 water results in greater promotion effect than UFB2 water

It is clearly indicated that the relationship of T_{50}-UFB1 is shorter than T_{50}-UFB2 and that both T_{50}-UFB1 and T_{50}-UFB2 are shorter than T_{50}-control as shown in Figure 2. T_{50}-UFB1, T_{50}-UFB2 and T_{50}-control were 13,7 h, 14,3 h and 16,5 h, respectively. This indicates an accelerated germination speed in both UFB sections according to their number concentration. The number concentration of UFB in UFB1 water was $8{,}5\times10^8$/ml and that in UFB2 water was $1{,}4\times10^8$/ml.

In this case, UFB2 water shall be chosen as a suitable UFB water. UFB1 water promotes also seed germination, however it is not the best choice as there can be a possible negative effect on the seeds. This is because of a possibility that there is another number concentration of UFB1' which has a number concentration lower than that of UFB1 and higher than that of UFB2 and that T_{50}-UFB1' is shorter than both T_{50}-UFB1 and T_{50}-UFB2. This means that UFB1' water contains UFBs being at nearly the upper limit of number concentration which can exhibit the greatest promotion effect of seed germination without any physiological damage. Under this assumed condition, the process indicated by A1 can be understood to mean a suppression process of germination. See Case 3 in 6.4 as a reference.

Figure 3 shows 95 % confidence intervals of T_{50} values of UFB1, UFB2 and control section. Concave curves show sum squared of residuals of T_{50}-UFB1, T_{50}-UFB2 and T_{50}-control. The ranges between concave curves and horizontal lines show 95 % confidence intervals. Since 95 % confidence interval of UFB1 water expressed by A1 does not overlap with that of UFB2 expressed by A2, there is a significant difference in T_{50}.



Key

○	data at control section
□	data at UFB1 section
+	data at UFB2 section
T	measurement time, expressed in hours
G	germination ratio, expressed in per cent
Ai	regression curve of germination ratio of seeds in UFBi water, i = 1 and 2
B	regression curve of germination ratio of seeds in control water
G_{max}	point to reach the maximum germination ratio, expressed in per cent
G_{50}	point to reach 50 % of maximum germination ratio, expressed in hours
T_{50}-control	crossing time with G_{50} and the curve of control section, expressed in hours
T_{50}-UFBi	crossing time with G_{50} and the curve of UFBi section, expressed in hours, i = 1 and 2

Figure 2 — Case 1: number concentrations of UFBs satisfying the relationships UFB1>UFB2 and T_{50}-UFB1<T_{50}-UFB2<T_{50}-control (Kobinkatagi)

