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Fine bubble technology — Agricultural applications —

Part 3: Guidelines for the minimum viable number concentration of ultrafine bubbles for promoting the germination of barley seeds

Technologie des fines bulles — Applications agricoles —

Partie 3: Lignes directrices relatives à la concentration minimale en nombre viable de bulles ultrafines pour favoriser la germination des graines d'orge

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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

The market for technologies using fine bubbles have been rapidly growing in many applications throughout the industrial, domestic and academic sectors. Most notably, the applications of ultrafine bubble (UFB) technology to the agricultural area are drawing very high interest as they are thought to be part of the key technologies improving the productivity and efficiency of agriculture. Consequently, this contributes to the promotion of the United Nations Sustainable Development Goals (SDGs) in terms of providing sufficient food and maintaining water resources on land, for example.

As part of the strategy for standardizing the agricultural applications of fine bubbles, ISO 23016-2 was published, describing the test method for promoting barley seed germination by application of a UFB water generation system. Although UFB number concentration is closely related to the promotion of barley seed germination, the effects of concentration were not specified in it.

For the practical application of UFB technology to seed germination, it is important to provide data specifying the minimum number concentration of UFB necessary for promoting seed germination. This data provides useful information to users in their selection of an appropriate UFB generation system.

This document describes the collected data indicating the experimental observations between various number concentrations of UFB and their promotion effects on barley seed germination.

Fine bubble technology — Agricultural applications —

Part 3: Guidelines for the minimum viable number concentration of ultrafine bubbles for promoting the germination of barley seeds

1 Scope

This document demonstrates guidelines for promoting the germination of barley seeds with a lower number concentration of ultrafine bubbles (UFB). This is achieved by taking the data concerning the germination ratio of barley seeds conforming to ISO 23016-2 as a starting point and then evaluating the minimum number concentration range of ultrafine bubble water necessary for promoting the effect on germination of barely seeds by changing germination time.

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

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 23016-2 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
median germination period
T_{50}
time where the inferred germination ratio marks 50 % of the maximum germination ratio

Note 1 to entry: See Reference [1].

3.2
storage period
time length when barley seeds are stored under 20 °C

3.3
dormancy
failure of an intact viable seed to complete germination under favourable conditions

Note 1 to entry: The related term 'dormancy breaking' describes the breaking of dormancy. See Reference [2].

4 Test subjects

The items subject to the test are air containing UFB water stored in bottles or other containers for preservation and transport, and the UFB generating system used to generate the UFB water. UFB water is generated by supplying raw water to the UFB generating system. Distilled water with a quality of A2[1)] or greater is used as raw water according to ISO 23016-2.

The size, quantity and concentration of UFB in UFB water are measured and an example of measured data is given in Annex A.

5 Judgement of significant difference in T_{50}

After determining the correlation curve shown in ISO 23016-2:2019, Formula (2), the sum squared of residual (SSR) and standard error (SE) around the time T_{50} are calculated. From this curve, the 95 % confidence interval of T_{50} is determined.

6 Seeds and measurement device

6.1 Seeds for germination test

Barley seeds stored longer than the period of dormancy breaking were used as the seeds for the germination test. Barley seeds are not obtained from a specific location, but can be obtained anywhere. It was not necessary for the seeds to be rinsed in water for use; the test was initiated using the dry seeds as supplied. The germination test was conducted at the constant temperature of 25 °C.

6.2 Measurement device for UFB size and concentration

The air containing UFB water was generated using the pressure dissolution system with the pressure just after the pressurizing pump at around 700 kPa and around 300 kPa at the saturator. These samples had their size and concentration measured using a particle tracking analysis instrument (see ISO 19430). The instrument used was a Nano Sight (Malvern Panalytical, UK)[2)], allowing a measuring range from 50 to 1 000 nm. The wavelength of laser light source was 635 nm, the power was 40 mW, and a camera with black and white charge coupled device (CCD) was used in conjunction with analysis software of NTA 3.1 Build 3.1.46. Preset parameters are given in Annex A. The measuring temperature was at a room temperature of around 22 °C.

6.3 Examination range of UFB number concentration

As the UFB generation system given in ISO 23016-2 stably generates UFB in the number concentration ranges from 10^7/ml, 10^8/ml and greater, the promotion effect in the lower region of number concentration of UFB was examined from between10^7/ml to 10^8/ml. The air UFB number concentration in this range is known to be within the range able to be measured by the commercially available device mentioned in 6.2.

7 Measurement data

7.1 Necessary storage period of barley seeds longer than that of dormancy breaking

As barley plants grown under cool temperatures produce highly dormant seeds, extended periods of after-ripening are required to break dormancy. For example, storage for 3 to 4 months is required to allow dormancy to decay[3]. It is hoped that the barley seeds to be used are stored longer than the

1) Electrical conductivity 0,1 mS/S (25 °C), total organic carbon (TOC) 0,5 mg-C/l or less, zinc 0,5 µg-Zn/l or less, silica 50 µg-SiO_2/l or less, chloride ions µg-Cl^-/l, sulphide ions µg-SO_4^{2-}/l.

2) "Nano Sight" is an example of a suitable product available commercially. This information is given for the convenience of users of this document and does not constitute an endorsement by ISO of this product.

period of dormancy breaking. The seeds used in this test described in this document were stored for 11 months or more, which means dormancy breaking seeds were used.

7.2 Germination promotion effects in association with each number concentration of UFB and the storage period of seeds

Every measurement result is shown in Table 1. Judging from evaluation of the median germination time (T_{50}), it is shown that there is a clear turning point for the deterioration of seeds' quality for a storage period between 34 and 38 months.

In terms of germination promotion effects, in all cases, the median germination time was $T_{50,UFB} < T_{50,C}$, (where "$_C$" represents the control section). This shows the improvement in germination speed when UFB is used. However, in sample No. 1 ($1{,}1\times10^7$/ml) and sample No. 3 ($3{,}5\times10^7$/ml), statistically significant differences were not seen. This is considered to have been caused by an excessively low number concentration being below the threshold required for the promotion of germination.

Meanwhile, the results with a number concentration of UFB about three times as high that of sample No. 1 under a similar storage period for the seeds are shown as sample No. 2 ($3{,}1\times10^7$/ml) and sample No. 3 ($3{,}5\times10^7$/ml). On the contrary to the case of sample No. 2, where there was a significant difference between $T_{50,C}$ and $T_{50,UFB,}$ a significant difference was not recognized in the case of sample No. 3. Therefore, it was judged that a statistically stable effect on the promotion of germination was not expected at this number concentration level.

Furthermore, when the number concentration increased, i.e., in the region of sample No. 4 to sample No. 11 (from $7{,}2\times10^7$/ml to $1{,}4\times10^9$/ml), the germination promotion effect with statistically significant difference was seen regardless of the storage period (seed quality)[4]. Seeds used from samples No. 1 to 4, and samples No. 8, 9 and 11 were shown to have high qualities, supported by $G_{max,C}$ (where "$_C$" represents the control section) being higher than 90 %. On the other hand, seeds from samples No. 5 to 7 and No. 10 were shown to have low quality, with $G_{max,C}$ being less than 65 %.

Table 1 — Germination promotion effect on barley seeds with UFB number concentration and storage period

Exponent	No.	Number concentration (/ml)	Storage duration (month)	$T_{50,C}$ (h)	$T_{50,UFB}$ (h)	$G_{max,C}$ (%)[a]	$G_{max,UFB}$ (%)[a]	Significant difference	$P \pm \Delta P$[b]
6	0	The UFB water with the bubble number concentration of the order of 10^6/ml did not show any germination promotion effect on the seeds.						NO	Not applicable
7	1	$1{,}1\times10^7$	25	20,4	19,6	92,0	97,0	NO	3,9 ± 4,2
	2	$3{,}1\times10^7$	26	20,7	18,9	95,0	92,0	YES	8,7 ± 1,7
	3	$3{,}5\times10^7$	25	20,4	18,7	92,0	95,0	NO	8,3 ± 3,9
	4	$7{,}2\times10^7$	11	14,6	13,0	92,0	94,0	YES	11,0 ± 1,6
8	5	$1{,}5\times10^8$	45	73,2	39,1	62,0	87,3	YES	46,6 ± 2,2
	6	$2{,}0\times10^8$	44	102,8	49,7	49,3	82,7	YES	51,7 ± 1,9
	7	$2{,}3\times10^8$	38	46,4	39,6	37,3	44,0	YES	14,7 ± 5,0
	8	$2{,}4\times10^8$	34	22,0	20,9	91,3	91,3	YES	5,0 ± 1,1
	9	$5{,}6\times10^8$	34	22,0	20,7	91,3	93,3	YES	5,9 ± 1,5
	10	$6{,}6\times10^8$	38	77,6	37,9	37,3	76,7	YES	51,2 ± 6,9
9	11	$1{,}4\times10^9$	27	15,2	13,2	99,3	97,3	YES	13,2 ± 1,8

[a] *G*: Germination ration, % (see ISO 23016-2).

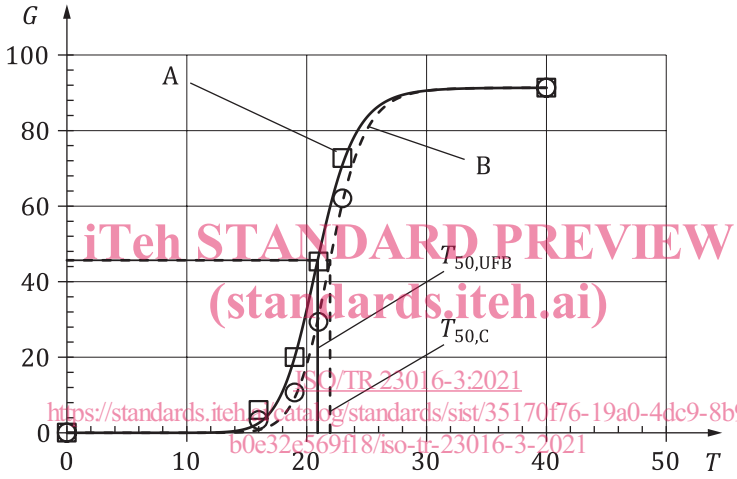
[b] *P*: UFB enabled efficiency (see ISO 23016-2).

7.3 Results of the data analyses

7.3.1 Typical example

7.3.1.1 Germination promotion effect on high-quality seeds

The result of analyzing sample No. 8 in Table 1 is shown in Figures 1, 2 and 3 as an example of the germination promotion effect by UFB on high-quality seeds (storage period of 34 months). The number concentration of UFB was 2,4 × 10^8/ml. Figure 1 is the result of regression analysis on the data using ISO 23016-2:2019, Formula (2), where the symbol □ shows the time course change of the germination ratio of seeds submerged in UFB water and the symbol ○ shows the time in control water. Figure 2 shows the time required to reach 50 % of the maximum germination ratio in percent (T_{50}) and Figure 3 shows 95 % confidence intervals of T_{50} in the UFB section and the control section, respectively. One plot shows the average value of 3 sets of data. Since the effect of UFB had no duplication at 95 % confidence interval (Figure 3), there was a significant difference in T_{50}. However, the final germination ratio had no difference, although the germination speed increased (T_{50} was shortened).



Key

○ data at control section

□ data at UFB section

A regression curve of germination ratio of seeds in UFB water

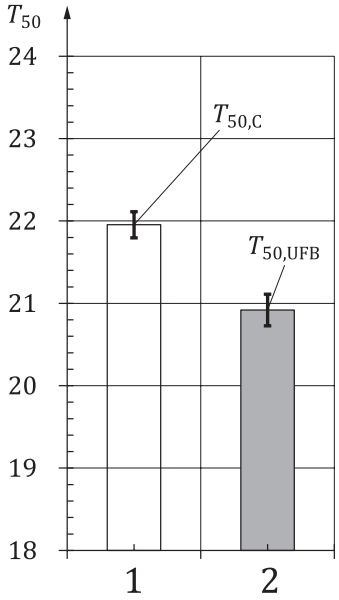
B regression curve of germination ratio of seeds in control water

G germination ratio, expressed in %

T measurement time, expressed in h

NOTE The keys shown in this figure are also used in Figure 4 to Figure 31 to indicate the same meanings.

Figure 1 — Promoting the germination of high-quality barley seeds by UFB (sample No. 8 in Table 1)



Key

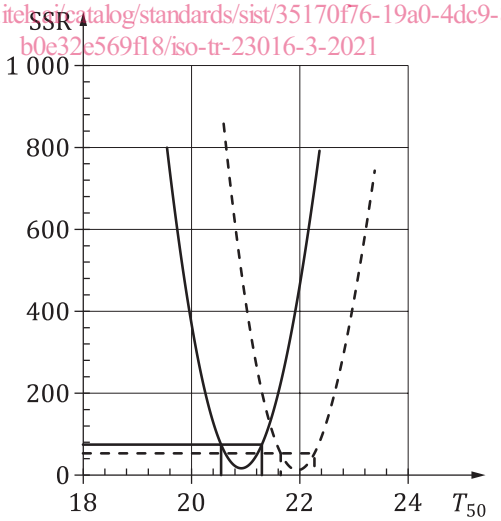
T_{50} time required to reach 50 % of maximum germination ratio

1 control water

2 UFB water

NOTE The keys shown in this figure are also used in Figure 5 to Figure 32 to indicate the same meanings.

Figure 2 — Time required to reach 50 % of maximum germination ratio in percent (sample No. 8 in Table 1)



Key

SSR sum squared of residual

_____ UFB of SSR

- - - - - control of SSR

NOTE The keys shown in this figure are also used in Figure 6 to Figure 33 to indicate the same meanings.

Figure 3 — 95 % confidential intervals of T_{50} both in UFB and control section of barley seeds of high quality (sample No. 8 in Table 1)

7.3.1.2 Germination promotion effect on low-quality seeds

The analysis result of sample No. 5 in Table 1 is shown in Figure 4, Figure 5 and Figure 6 as an example of the germination promotion effect of UFB for low-quality seeds (storage period of 45 months). The number concentration of UFB was 1,5 × 10^8/ml. Regarding low-quality seeds, a remarkable improvement effect in the final germination ratio and accelerated germination speed in the UFB section was shown (Figure 4). Furthermore, as shown in Figure 6, there was also a great gap between the UFB and control section at a 95 % confidence interval, and an outstandingly significant difference in T_{50} was recognized.

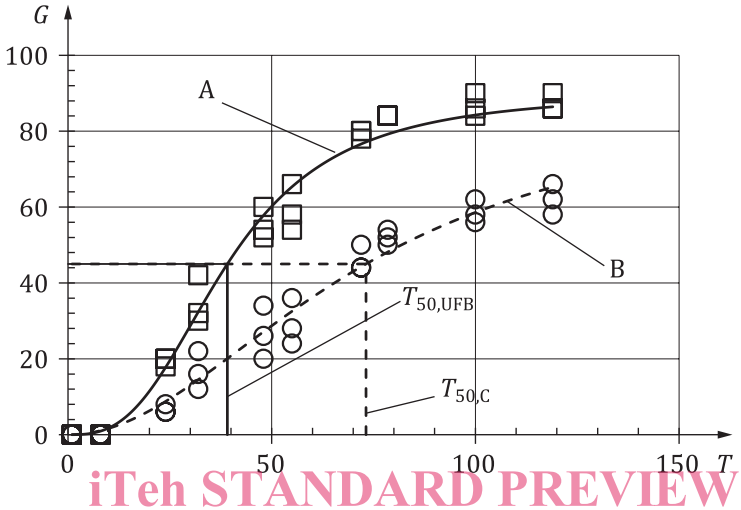
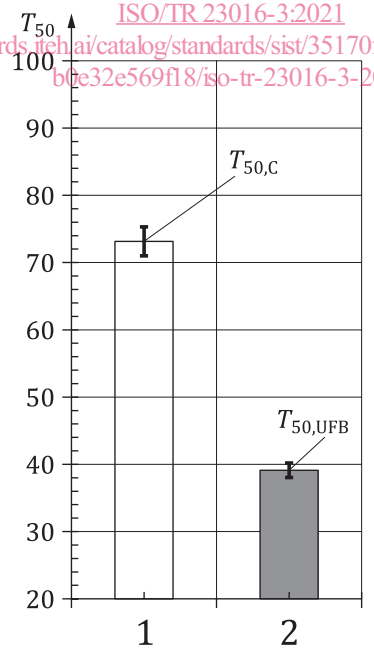


Figure 4 — Promoting the germination of low-quality barley seeds by UFB (sample No. 5 in Table 1)



Key

1 control

2 UFB

Figure 5 — Time required to reach 50 % of maximum germination ratio in percent (sample No. 5 in Table 1)