Technical Report

ISO/TR 23016-5

First edition
2024-08

Fine bubble technology — Agricultural applications —

Part 5: Practical data collection to promote the germination of typical vegetable seeds using ultrafine bubbles

Technologie des fines bulles — Applications agricoles —

Partie 5: Collecte de données pratiques pour favoriser la germination de graines de légumes types par l'utilisation de bulles ultrafines

Reference number
ISO/TR 23016-5:2024(en)

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Published in Switzerland

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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 has been rapidly growing in many applications throughout the industrial, domestic, and academic sectors. Especially, the application of ultrafine bubble (UFB) technology to the agricultural area is arousing great interest. It is thought to be one of the advanced technologies improving the productivity and efficiency of agriculture, thereby contributing to the United Nations Sustainable Development Goals (SDGs) by means of providing sufficient food and maintaining water resources on land, as an example.

In this context, ISO 23016-2:2019 has been published, describing the test method for promoting barley seed germination by application of a UFB water generation system. Although the promotion of germination of barley seed is closely related to UFB number concentration, effects of the concentration were not specified at that time.

Thus, in order to provide users with a guideline for selecting an appropriate UFB generation system, ISO/TR 23016-3:2021 has been published to indicate the minimum viable number concentration of ultrafine bubbles that promotes the germination of barley seeds.

Furthermore, based on the continual accumulation of data revealing positive and negative effects of UFB on germination depending on the variety of barley seeds, ISO 23016-4 has been published to provide a method to evaluate the UFB number concentration ensuring the promotion of germination of the barley seeds without taking into account their varieties.

This document describes the data collected from the experimental observations on applying UFB water to promote the germination of vegetable seeds, based on ISO 23016-2, ISO/TR 23016-3 and ISO 23016-4. It was developed to support the application of the UFB technology to vegetables specially grown in hydroponic culture system, which market is quite large and still growing.

Fine bubble technology — Agricultural applications —

Part 5: Practical data collection to promote the germination of typical vegetable seeds using ultrafine bubbles

1 Scope

This document provides practical data collection of promoting the germination of typical vegetable seeds by applying ultrafine bubbles (UFBs) within the effective number concentrations for barley seeds specified in ISO 23016-2, ISO/TR 23016-3 and ISO 23016-4. While the application of UFB to barley seeds is systematically standardized, reports on UFB application to vegetable seeds germination are scattered worldwide. Therefore, this document intends to illustrate the effectiveness of UFBs to promote the germination of vegetable seeds depending on their response to light, i.e.:

a) require light to germinate (positive photoblastic),

b) require darkness to germinate (negative photoblastic), and

c) neutral to light[1].

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*

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 23016-4, *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*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in ISO 23016-2, ISO/TR 23016-3 and ISO 23016-4 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/

4 Test subjects

The items subjected to the test are air UFB water stored in bottles or other containers for preservation and transport in accordance with ISO 21255, 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 Grade 2 according to ISO 3696 or greater is used as raw water that is distilled water supplied as a raw material for both UFB water and control water according to ISO 23016-2:2019, 3.6.

The size, quantity and concentration of UFB in UFB water were measured. Examples of measured data are given in Annex A.

5 Judgement of significant difference in T_{50in}

After determining the correlation curve using 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. The results are shown in Figures B.1 to B.7 in Annex B.

6 Seeds and measurement device

6.1 Seeds for germination test

The following vegetable seeds of different responses to light were used as the seeds for the germination test:

a) require light to germinate (positive photoblastic),

b) require darkness to germinate (negative photoblastic), and

c) neutral to light (neutral photoblastic)[1].

It is not necessary to rinse the seeds in water before use; the test is 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 UFB generating system is capable of supplying the UFB water described in ISO 23016-2. The air UFB water samples had their size and concentration measured using a particle tracking analysis instrument (see ISO 19430:2016). The size, quantity and concentration of UFB in UFB water were measured. Measurements were carried out at a room temperature around 22 °C.

6.3 Examination range of UFB number concentration for vegetable seeds and adjustment of dissolved oxygen concentration (DO)

As the UFB generation system given in ISO 23016-2 stably generates UFB in the number concentration ranging from 10^7/ml, 10^8/ml and greater, the UFB in the range from 10^7/ml to 10^8/ml was applied to vegetable seeds to ensure the effect of germination promotion. The air UFB number concentration in this range is known to be within the range that can be measured by the commercially available measurement device in 6.2.

Although an example of size distribution and number concentration of UFB used for barley seed germination is given in ISO/TR 23016-3:2021, Annex A, this case illustrates small-scale fluctuation. In this document, examples with fluctuation to some extent are shown in Figure A.1 for UFB number concentration of $1{,}5 \times 10^7$/ml and Figure A.2 for UFB number concentration of $1{,}1 \times 10^8$/ml.

The DO of water containing UFBs was adjusted to be the same as that of control water by introducing air and nitrogen gases through a mixed-gas flow regulator[2] in order to observe only the effect of UFBs on vegetable seed germination by cutting out the effect of DO.

7 Test data

7.1 General

This document covers the following studies:

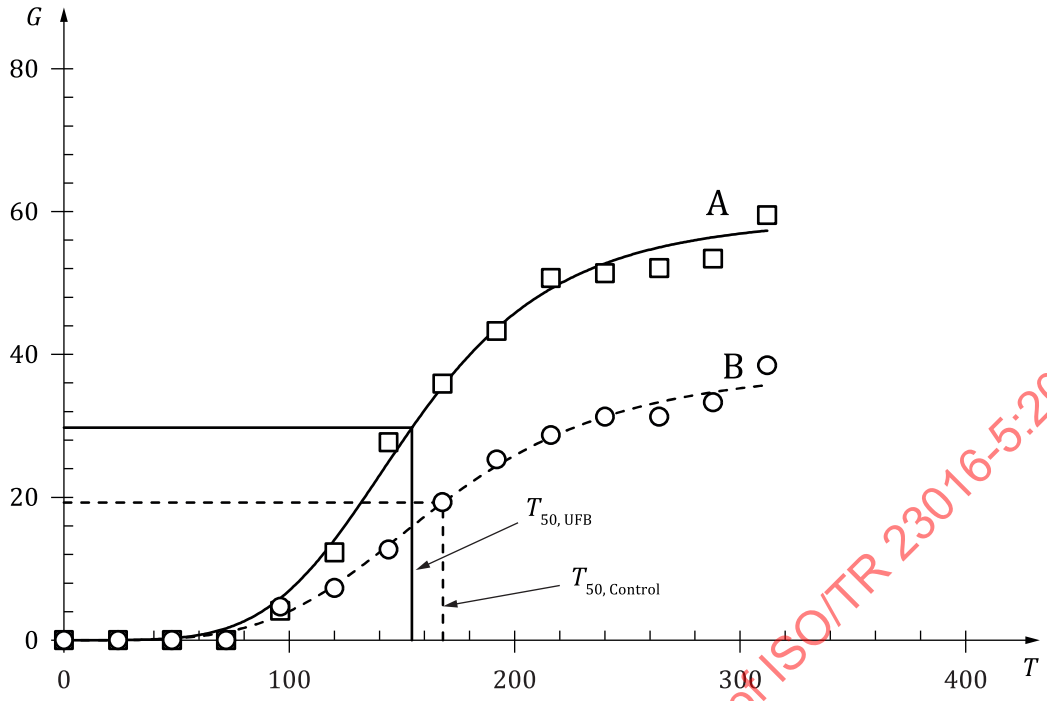
— The promotion effect on the germination for each of three types of seeds, which is described in 7.2 to 7.4.

— The effects of DO and number concentration of UFB on the germination promotion, which are described in 7.5 and 7.6.

— An effect of the seed size on the germination with using UFB is described in 7.7. In order to confirm the significant differences of T_{50} values between UFB and control sections, 95 % confidence intervals of T_{50} of UFB and control section were calculated and shown as Figures B.1 to B.7, each of those is relating to Figures 1 to 7.

7.2 Carrot seeds of positive photoblastic

The analysis result of germination processes of carrot seeds is shown in Figure 1 indicating the germination promotion effect of UFB. The number concentration of UFB was 4.4×10^7/ml. Dissolved oxygen concentration (DO) was adjusted to 8,0 mg/l for both UFB and control water. A remarkable improvement at the final germination ratio of 59,5 % in UFB section was observed compared to 38,5 % for control section. Furthermore, 95 % confidence intervals of T_{50} of UFB and control section were not overlapped as shown in Figure B.1. Thus, the promotion effect of UFB was verified on the germination of carrot seed which requires light for germination (positive photoblastic).



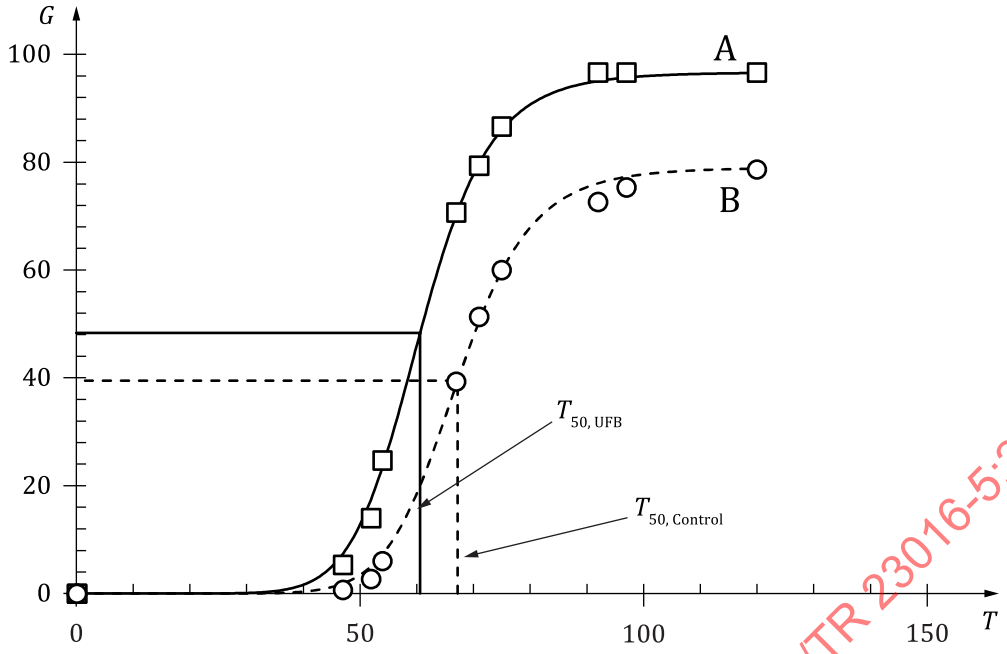
Key

○	data at control section
□	data at UFB section
T	measurement time, expressed in hours
G	germination ratio, expressed in per cent
A	regression curve of germination ratio of seeds in UFB water
B	regression curve of germination ratio of seeds in control water
$T_{50, Control}$	crossing time with 50 % of maximum germination ratio and the curve of control section, expressed in hours
$T_{50, UFB}$	crossing time with 50 % of maximum germination ratio and the curve of UFB section, expressed in hours

Figure 1 — Promotion effect of UFB with number concentration of 4,4 × 10^7/ml on germination of carrot seeds when DO of both control and UFB water is adjusted to 8,0 mg/l

7.3 Tomato seeds of negative photoblastic

The analysis result of germination processes of tomato seeds is shown in Figure 2 indicating the germination promotion effect of UFB. The number concentration of UFB was 1,1 × 10^8/ml. Dissolved oxygen concentration (DO) was adjusted to 7,6 mg/l to 7,7 mg/l for both UFB and control water. A remarkable improvement in the final germination ratio of 97,0 % for UFB section was observed compared to 79,0 % for control section. Furthermore, 95 % confidence intervals of T_{50} of UFB and control section were not overlapped as shown in Figure B.2. Thus, the promotion effect of UFB was verified on the germination of tomato seed which requires darkness to germinate (negative photoblastic).



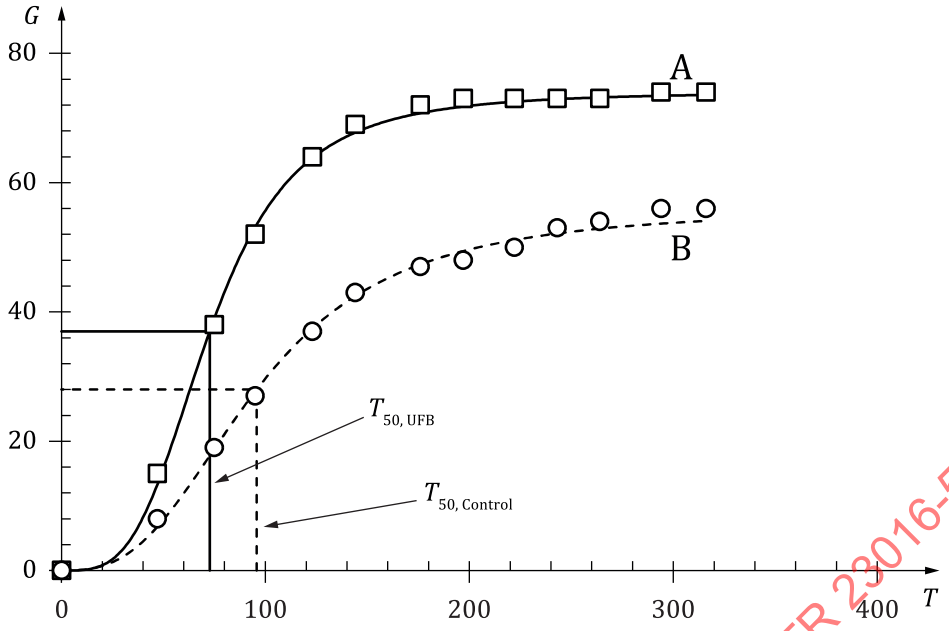
Key

○	data at control section
□	data at UFB section
T	measurement time, expressed in hours
G	germination ratio, expressed in per cent
A	regression curve of germination ratio of seeds in UFB water
B	regression curve of germination ratio of seeds in control water
$T_{50, Control}$	crossing time with 50 % of maximum germination ratio and the curve of control section, expressed in hours
$T_{50, UFB}$	crossing time with 50 % of maximum germination ratio and the curve of UFB section, expressed in hours

Figure 2 — Promotion effect of UFB with number concentration of 1,1 × 10^8/ml on germination of tomato seeds when both DO of both control and UFB water is adjusted to 7,6 mg/l to 7,7 mg/l

7.4 Spinach seeds of neutral photoblastic

The analysis result of germination processes of spinach seeds is shown in Figure 3 indicating the germination promotion effect of UFB. The number concentration of UFB was 1,1 × 10^8/ml. Dissolved oxygen concentration (DO) was adjusted to 7,8 mg/l to 7,9 mg/l for both UFB and control water. A remarkable improvement in the final germination ratio of 74,0 % for UFB section was observed compared to 56,0 % for control section. Furthermore, 95 % confidence intervals of T_{50} of UFB and control section were not overlapped as shown in Figure B.3. Thus, the promotion effect of UFB was verified on the germination of spinach seed which is neutral to light (neutral photoblastic).



Key

○	data at control section
□	data at UFB section
T	measurement time, expressed in hours
G	germination ratio, expressed in per cent
A	regression curve of germination ratio of seeds in UFB water
B	regression curve of germination ratio of seeds in control water
$T_{50, Control}$	crossing time with 50 % of maximum germination ratio and the curve of control section, expressed in hours
$T_{50, UFB}$	crossing time with 50 % of maximum germination ratio and the curve of UFB section, expressed in hours

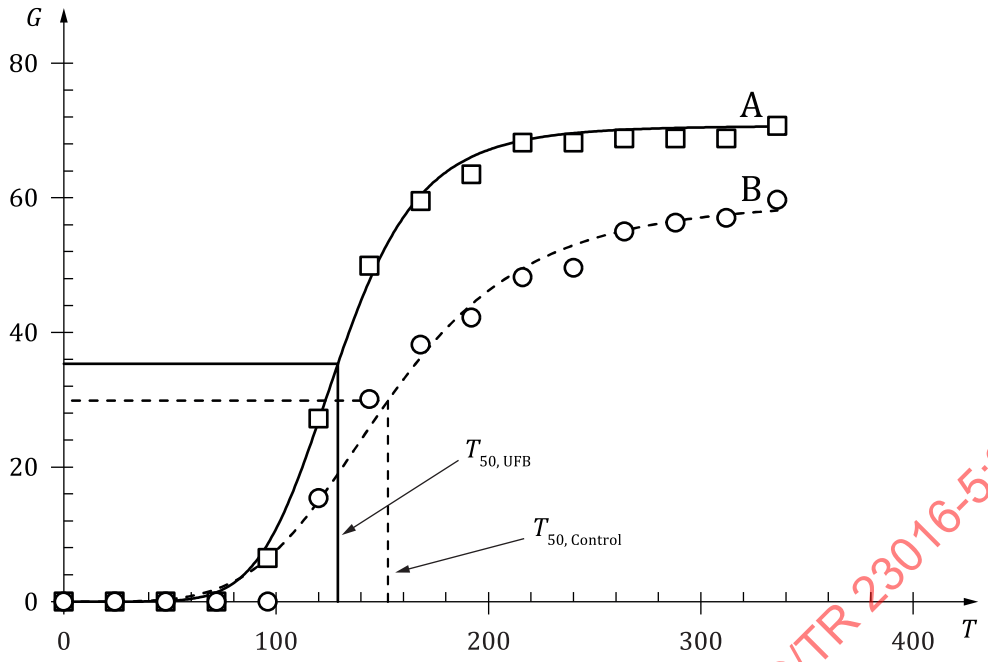
Figure 3 — Promotion effect of UFB with number concentration of 1,1 × 10^8/ml on germination of spinach seeds when both DO of both control and UFB water is adjusted to 7,8 mg/l to 7,9 mg/l

7.5 Effect of DO (Dissolved oxygen concentration)

The analysis result of germination processes of carrot seeds is shown in Figure 4 indicating the germination promotion effect of UFB under the condition that dissolved oxygen concentration (DO) was adjusted to 12,0 mg/l for both UFB and control water. The number concentration of UFB was 4,3 × 10^7/ml. A remarkable improvement in the final germination ratio of 70,7 % for UFB section was observed compared to 59,7 % for control section. Furthermore, 95 % confidence intervals of T_{50} of UFB and control section were not overlapped as shown in Figure B.4.

Comparing the condition of the result in 7.2 (Figure 1) and that in this subclause (Figure 4), the variety of seed is same and there is no clear difference in number concentration between 4,4 × 10^7 /ml (see 7.2) and 4,3 × 10^7 /ml (see 7.4). Only the values of DO differ with 8,0 mg/ml for Figure 1 and 12,0 mg/l for Figure 4. Therefore, the DO levels are thought to account for the difference in germination process.

In fact, the maximum germination ratio of control section in Figure 1 with DO = 8,0 mg/l is 38,5 % and that in Figure 4 with DO = 12,0 mg/l is 59,7 %. This increase in the germination ratio is due to the difference in DO. However, in the case of UFB section, the difference in the germination ratio is exceeded. Namely, the maximum germination ratio of UFB section (Figure 1) with DO = 8,0 mg/l is 59,5 % and that in Figure 4 with DO = 12,0 mg/l is 70,7 %. These experimental facts indicated that the UFB's promotion effect on seed germination appeared irrespective of the DO value.



Key

○	data at control section
□	data at UFB section
T	measurement time, expressed in hours
G	germination ratio, expressed in per cent
A	regression curve of germination ratio of seeds in UFB water
B	regression curve of germination ratio of seeds in control water
$T_{50, Control}$	crossing time with 50 % of maximum germination ratio and the curve of control section, expressed in hours
$T_{50, UFB}$	crossing time with 50 % of maximum germination ratio and the curve of UFB section, expressed in hours

Figure 4 — Promotion effect of UFB with number concentration of 4,3 × 10^7/ml on germination of carrot seeds when DO of both control and UFB water is adjusted to 12,0 mg/l

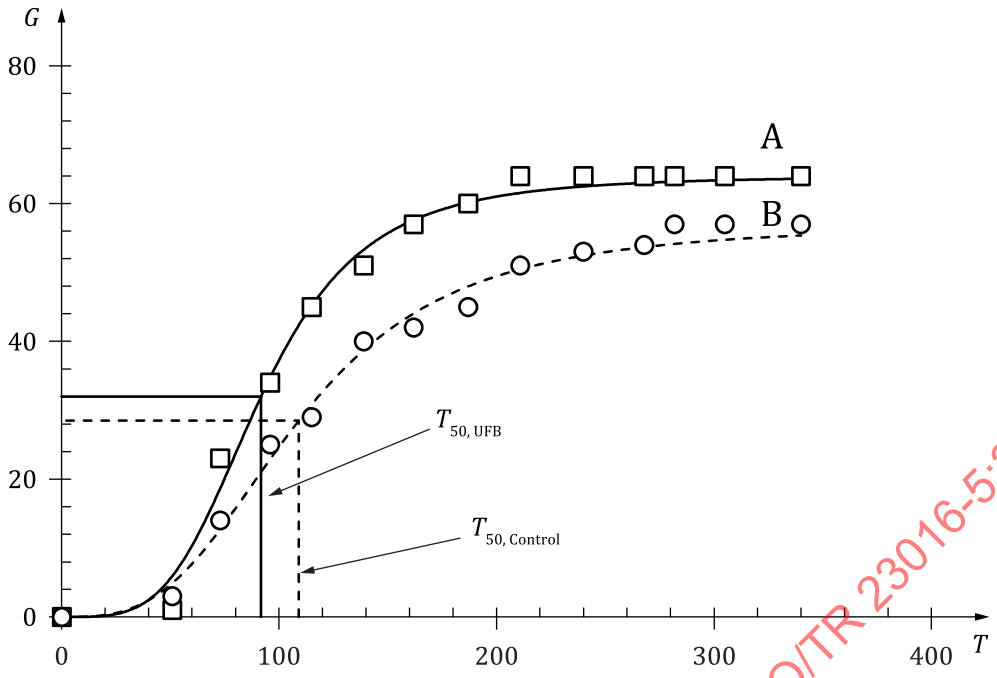
7.6 Effect of UFB number concentration

The analysis result of germination processes of spinach seeds is shown in Figure 5 indicating the germination promotion effect of UFB with a number concentration of UFB of 1,5 × 10^7/ml. The dissolved oxygen concentration (DO) was 7,7 mg/l for both UFB and control water. An improvement in the final germination ratio of 64,0 % for UFB section was observed compared to 57,0 % for control section. Furthermore, 95 % confidence intervals of T_{50} of UFB and control section were not overlapped as shown in Figure B.5.

Comparing the condition of the result in 7.4 (Figure 3) and that in this subclause (Figure 5), the variety of seed is same and there is no clear difference in the values of DO as 7,8 mg/l to 7,9 mg/l for Figure 3 (see 7.4) and 7,7 mg/l for Figure 5 (see 7.6).

The only marked difference lies in the values of UFB number concentration of 1,1 × 10^8/ml for Figure 3 and of 1,5 × 10^7/ml for Figure 5. Therefore, the number concentration levels are thought to account for the differences in germination process. In fact, the maximum germination ratios of control sections were very similar, i.e. 56,0 % for Figure 3 and 57,0 % for Figure 5. In contrast to this, the maximum germination ratio was promoted to 74,0 % with UFB number concentration of 1,1 × 10^8/ml (Figure 3) and 64,0 % with UFB number concentration of 1,5 × 10^7/ml (Figure 5).

These experimental facts indicated that higher UFB number concentration caused a noticeable germination promotion under similar values of DO.



Key

○	data at control section
□	data at UFB section
T	measurement time, expressed in hours
G	germination ratio, expressed in per cent
A	regression curve of germination ratio of seeds in UFB water
B	regression curve of germination ratio of seeds in control water
$T_{50, Control}$	crossing time with 50 % of maximum germination ratio and the curve of control section, expressed in hours
$T_{50, UFB}$	crossing time with 50 % of maximum germination ratio and the curve of UFB section, expressed in hours

Figure 5 — Promotion effect of UFB with number concentration of 1,5 × 10^7/ml on germination of spinach seeds when DO of both control and UFB water is adjusted to 7,7 mg/l

7.7 Presence or absence of seed size effect on the promotion by UFB

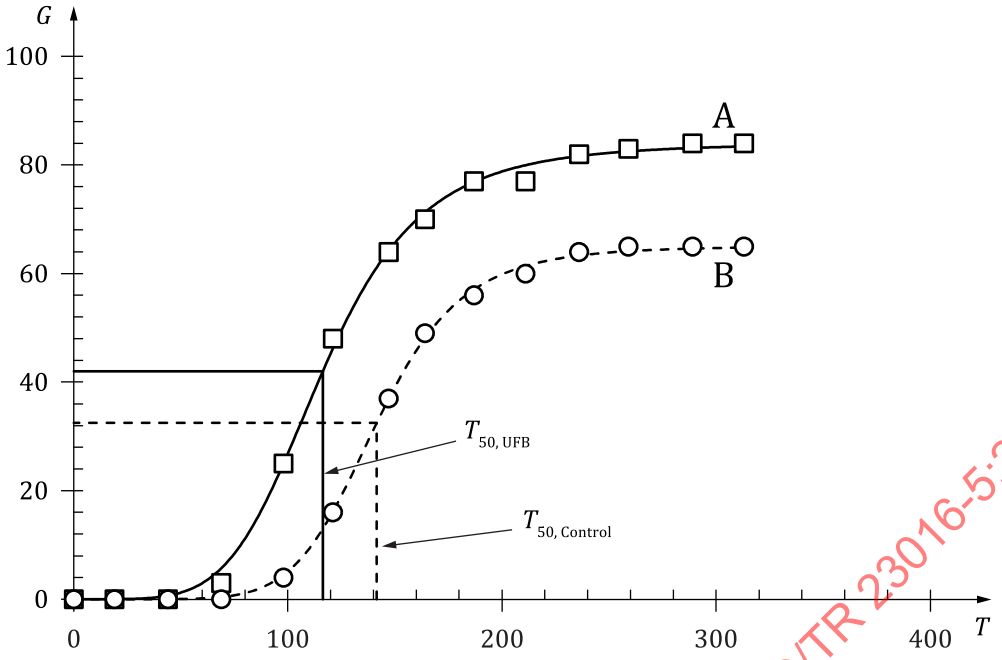
7.7.1 General

There are papers describing that large seeds have positive effect on germination comparing to small seeds for maize seed,[3] oat seed[4] and redbud seed,[5] while another paper describes that there is no significant differences between the size of wheat seed.[6] This information indicates the effect of size on germination appears depending on the seed type.

However, it is not known whether the promotion effect of UFB on germination is affected by seed size or not. In order to clarify this point, carrot seed, which is known as a seed of low germination ratio among vegetable seeds, was taken as a sample to investigate if seed size affects the UFB's promotion effect.

7.7.2 Carrot seed of comparatively large size

The analysis result of germination processes of carrot seeds of comparatively large size is shown in Figure 6 indicating the germination promotion effect of UFB. The number concentration of UFB was 1,1 × 10^8/ml. Dissolved oxygen concentration (DO) was adjusted to 7,6 mg/l to 7,7 mg/l for both UFB and control water. A remarkable improvement in the final germination ratio of 84,0 % for UFB section was observed compared to 65,0 % for control section. Furthermore, 95 % confidence intervals of T_{50} of UFB and control section were not overlapped as shown in Figure B.6. Thus, the promotion effect of UFB was verified on the germination.



Key

○	data at control section
□	data at UFB section
T	measurement time, expressed in hours
G	germination ratio, expressed in per cent
A	regression curve of germination ratio of seeds in UFB water
B	regression curve of germination ratio of seeds in control water
$T_{50, Control}$	crossing time with 50 % of maximum germination ratio and the curve of control section, expressed in hours
$T_{50, UFB}$	crossing time with 50 % of maximum germination ratio and the curve of UFB section, expressed in hours

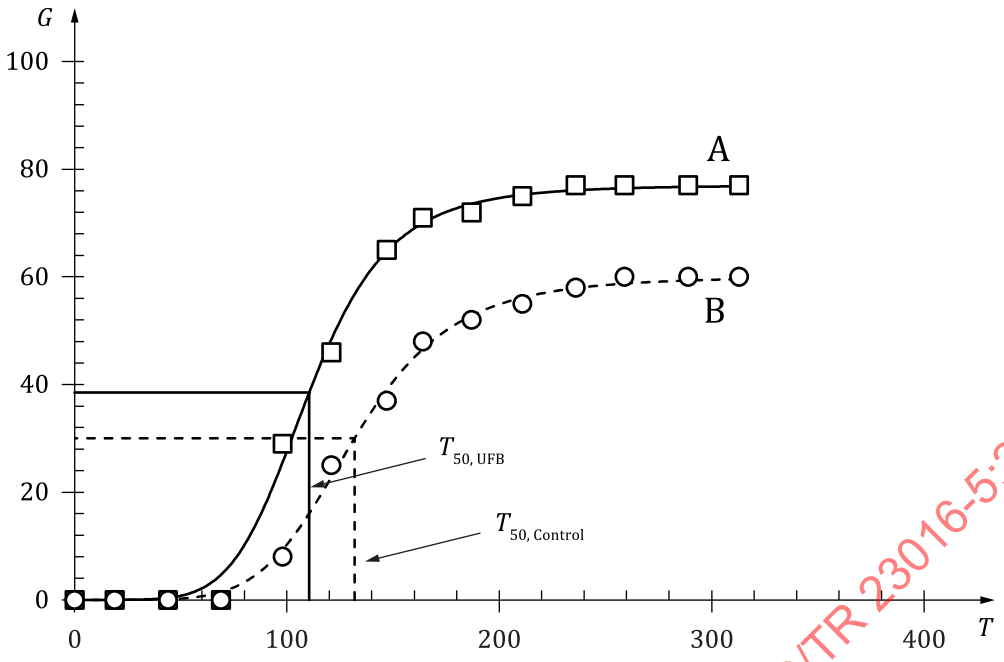
Figure 6 — Promotion effect of UFB with number concentration of 1,1 × 10^8/ml on germination of carrot seeds of comparatively large size when DO of both control and UFB water is adjusted to 7,6 mg/l to 7,7 mg/l

7.7.3 Carrot seed of comparatively small size

The analysis result of germination processes of carrot seeds of comparatively small size is shown in Figure 7 indicating the germination promotion effect of UFB. The number concentration of UFB was 1,1 × 10^8/ml. Dissolved oxygen concentration (DO) was adjusted to 7,6 mg/l to 7,7 mg/l for both UFB and control water. A remarkable improvement in the final germination ratio of 77,0 % for UFB section was observed compared to 60,0 % for control section. Furthermore, 95 % confidence intervals of T_{50} of UFB and control section were not overlapped as shown in Figure B.7. Thus, the promotion effect of UFB was verified on the germination.

Comparing with the condition of the result observed in 7.7.2 (Figure 6), there is no difference neither in the value of DO nor in the value of number concentration.

The only difference is the seed size, large and small. The seed size affected the final germination ratio of both UFB and control sections, i.e. 84,0 % of UFB section and 65,0 % of control section in the case of large seed size and 77,0 % of UFB section and 60,0 % of control section. As expected, large size seeds showed higher germination ratio for both UFB and control section than that of small size seeds. The extent of seed size effect on the promotion by UFB cannot be evaluated only with this result. However, UFB's promotion effect on seed germination stably appears without relation to seed size.



Key

○	data at control section
□	data at UFB section
T	measurement time, expressed in hours
G	germination ratio, expressed in per cent
A	regression curve of germination ratio of seeds in UFB water
B	regression curve of germination ratio of seeds in control water
$T_{50, Control}$	crossing time with 50 % of maximum germination ratio and the curve of control section, expressed in hours
$T_{50, UFB}$	crossing time with 50 % of maximum germination ratio and the curve of UFB section, expressed in hours

Figure 7 — Promotion effect of UFB with number concentration of 1,1 × 10^8/ml on germination of carrot seeds of comparatively small size when DO of both control and UFB water is adjusted to 7,6 g/ml to 7,7 mg/l

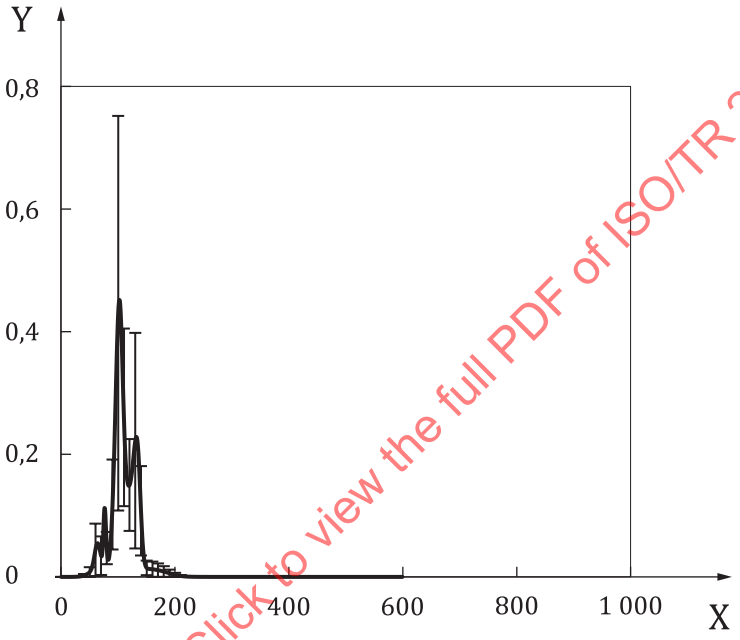
8 Suggestion

The DO of water containing UFBs was adjusted to be the same as that of control water by introducing air and nitrogen gases through a mixed-gas flow regulator, as described in 6.3, in order to observe only the effect of UFBs on vegetable seed germination by cutting out the effect of DO. However, in a practical use, DO of UFB water is normally higher than that of control water when DO is not specially adjusted. As higher DO induces higher germination ratio as confirmed in 7.5, it is suggested to use UFB water without adjustment of DO to attain a favourable germination promotion.

Annex A
(informative)

Example of measured UFB data of size distribution and number concentration of UFB used in this document

Examples of measured data with some extent of fluctuations normally observed from air UFB water sample in the number concentration range of 10^7/ml are shown in Figure A.1 and Table A.1, and those in the range of 10^8/ml are shown in Figure A.2 and Table A.2. Another example is also provided in ISO/TR 23016-3 as a reference.



Key

X size (nm)

Y number concentration (× 10^6 particles/ml)

Figure A.1 — Averaged size distribution of 3 measurements with standard errors for UFB number concentration of 1,5 × 10^7/ml

Table A.1 — Stats data of 3 measurements

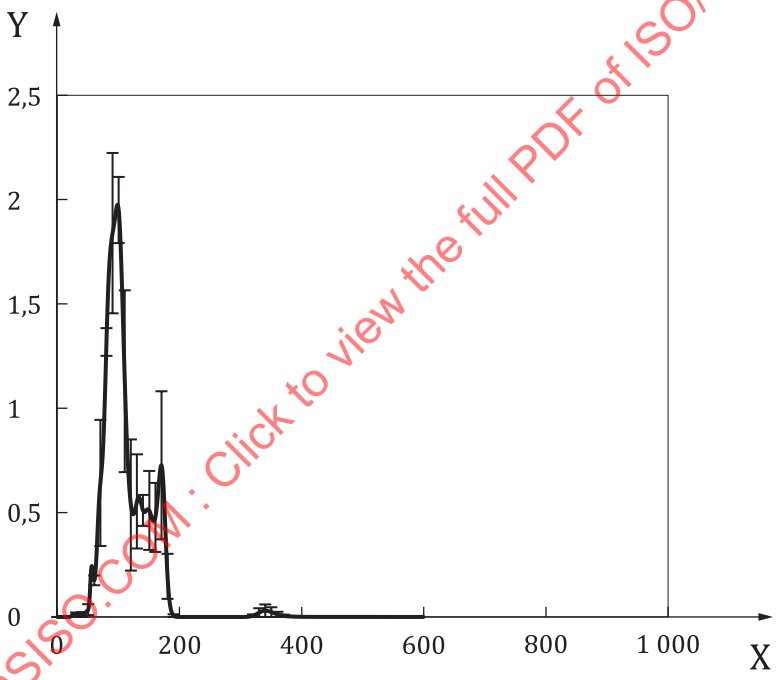
	Mean ± Standard errors
Mean [a]	110,3 ± 5,8 nm
Mode	112,5 ± 10,0 nm
SD [b]	21,8 ± 7,6 nm
D10 [c]	77,3 ± 9,0 nm
D50 [c]	110,2 ± 8,5 nm
D90 [c]	137,6 ± 13,4 nm
Concentration [d]	$1,5 \times 10^7 \pm 0,3 \times 10^7$/ml

[a] Average diameter of all particles detected

[b] Standard deviation

[c] Frequency which reaches to given value of total particle number

[d] Mean ± Standard error



Key

X size (nm)

Y number concentration (× 10^6 particles/ml)

Figure A.2 — Averaged size distribution of 3 measurements with standard errors for UFB number concentration of 1,1 × 10^8/ml

Table A.2 — Stats data of 3 measurements

	Mean ± Standard error
Mean [a]	110,7 ± 4,1 nm
Mode	92,1 ± 4,0 nm
SD [b]	35,6 ± 6,1 nm
D10 [c]	76,1 ± 1,2 nm
D50 [c]	100,8 ± 2,6 nm
D90 [c]	161,0 ± 6,5 nm
Concentration [d]	$1,1 \times 10^8 \pm 0,2 \times 10^8$/ml

[a] Average diameter of all particles detected

[b] Standard deviation

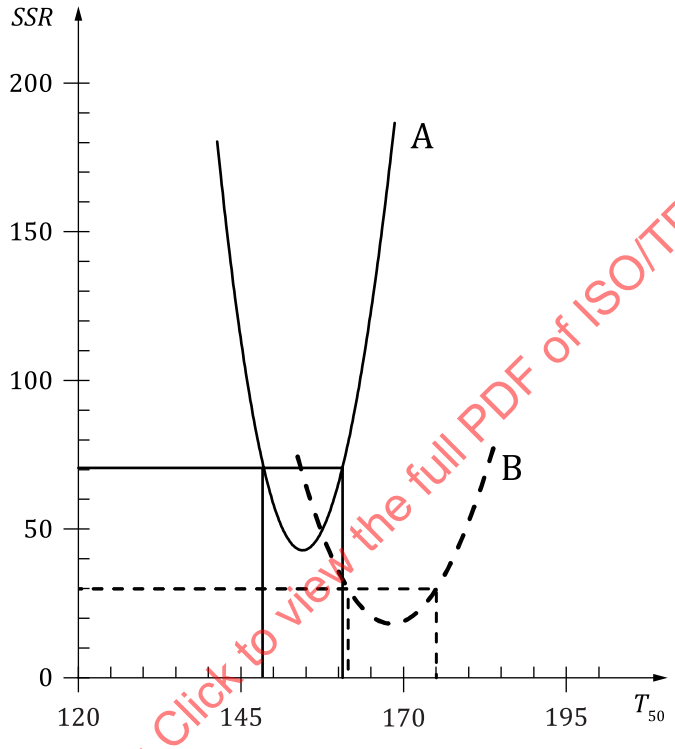
[c] Frequency which reaches to given value of total particle number

[d] Mean ± Standard error

Annex B
(informative)

95 % confidence intervals of T_{50} values corresponding to Figures 1 to 7

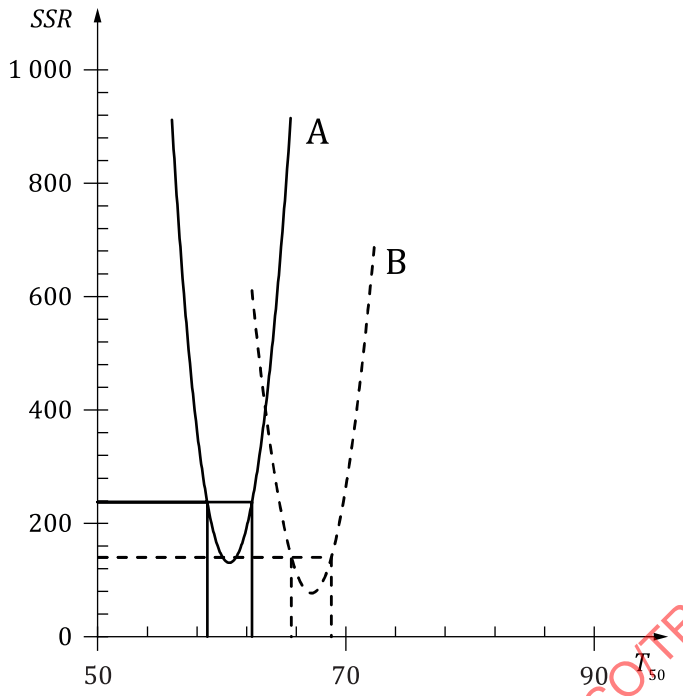
The promotion effect of UFB on vegetable seed germination shown in Figures 1 to 7 is verified statistically by the non-overlapping of the 95 % confidence intervals of T_{50} in UFB and control section. The 95 % confidence intervals of T_{50} corresponding to Figures 1 to 7 are given in Figures B.1 to B.7, respectively.



Key

SSR	sum squared of residual
T_{50}	crossing time with 50 % of maximum germination ratio and the regression curves in Figure 1, expressed in hours
A	SSR of $T_{50,\ UFB}$
B	SSR of $T_{50,\ Control}$

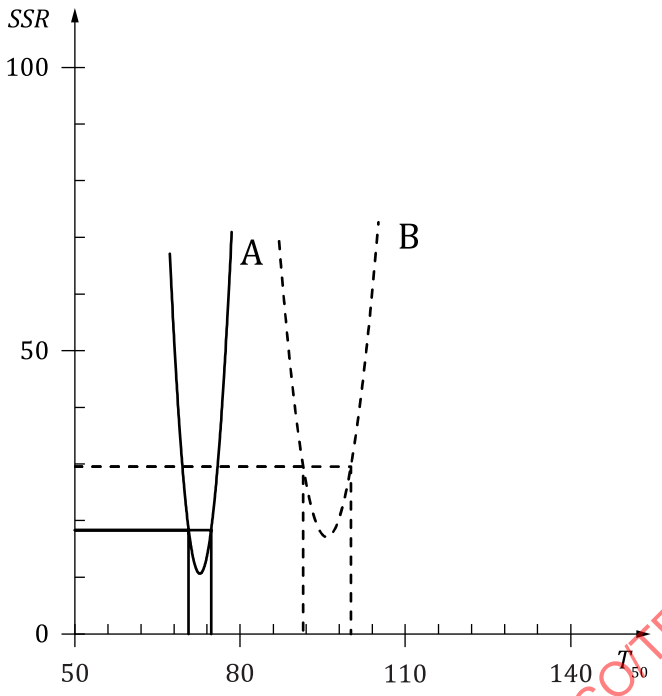
Figure B.1 — 95 % confidence intervals of T_{50} corresponding to Figure 1 (Carrot seeds at DO = 8,0 mg/l)



Key

SSR	sum squared of residual
T_{50}	crossing time with 50 % of maximum germination ratio and the regression curves in Figure 2, expressed in hours
A	SSR of $T_{50,\ UFB}$
B	SSR of $T_{50,\ Control}$

Figure B.2 — 95 % confidence intervals of T_{50} corresponding to Figure 2 (Tomato seeds at DO = 7,6 mg/l to 7,7 mg/l)



Key

SSR	sum squared of residual
T_{50}	crossing time with 50 % of maximum germination ratio and the regression curves in Figure 3, expressed in hours
A	SSR of $T_{50,\ UFB}$
B	SSR of $T_{50,\ Control}$

Figure B.3 — 95 % confidence intervals of T_{50} corresponding to Figure 3 (Spinach seeds at DO = 7,8 mg/l to 7,9 mg/l)