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Fine bubble technology — Agricultural applications —

Part 1: Test method for evaluating the growth promotion of hydroponically grown lettuce

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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.

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Introduction

The recent progress in the application of fine bubble technology exhibits successes in the various technical fields such as environmental technology in water and washing and cleaning technology for mechanical engineering. The applications for agro- and aqua- farming and food industrial field also draw high interests of markets in view of fine bubble enabled performance in enhancing growth of agro- and aqua- products, improving their quality, saving resources for farming and ensuring safety of the food products. Various industries engaged in such products are introducing the fine bubbles to their farming field by applying fine bubble generating systems, eventually creating new market for the generating systems.

However, since technology transfer from fine bubble technology to technology fields of agro- and aqua-farming and food industries is not well supported by common understanding of the fine bubbles or their generating technology, results of evaluation on fine bubble enhanced performance cannot be accepted commonly by both generating system suppliers and its users at the transaction scene. Furthermore, a variety of agro- and aqua- farming products makes it difficult to adopt a systematic approach for selection and application of generating systems.

The performance evaluation based on objective evidence resulting from standardized procedures is intended to bridge the two technologies and facilitate diverse fields of applications for fine bubble technology in the global market. In order to accelerate sound global market formation, development of test procedures is urgently demanded by both technology stakeholders.

This document is intended to meet these needs by specifying the test procedure to be applied to the generating system for agro- and aqua- farming and food industries uses. The evaluation is made by applying fine bubble water generated by the object system to lettuce and by measuring its growth. The product, lettuce, is globally accepted and the yielded test data represents the performance of the tested system over other products in such major product family as, for example, leaf vegetable. The growth process of lettuce is much simpler than other vegetables making the measuring process much easier in the test procedure. The specified test conditions, namely the environment for growth, are also easy to be controlled allowing many testing plants globally available. The parameter measured is the change in the harvested mass of lettuces with application of fine bubbles compared to that without application in a specified period of growth.

Since the performance in terms of parameters is improving rapidly as the technology evolves, the quantitative criteria for the testing are not specified in this document.

Fine bubble technology — Agricultural applications —

Part 1: Test method for evaluating the growth promotion of hydroponically grown lettuce

1 Scope

This document specifies a test method for evaluating the effect of fine bubble water on the growth promotion of hydroponically grown lettuce by estimating the incremental gain in mass of the stems and leaves over a specified growth period.

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
plant factory
facilities which allow systematic growth and production of plants where the internal environment conditions, e.g. temperature, carbon dioxide, and liquid fertilizer are controlled

3.2
fine bubble generating system
system which mechanically generates fine bubbles (bubbles less than 100 µm in diameter) using water and air

3.3
fine bubble water
water including fine bubbles and used in *plant factories* (3.1)

3.4
raw water
tap water or water with equivalent quality level provided to produce *fine bubble water* (3.3) and used as the control water for the reference

3.5
fine bubble section
area to grow plants using *fine bubble water* (3.3)

Note 1 to entry: For the purpose of this document, plants grown are lettuces.

3.6
control section
area to grow plants using *raw water* (3.4) as a reference for *fine bubble section* (3.6)

Note 1 to entry: For the purpose of this document, plants grown are lettuces.

3.7
liquid fertilizer
liquid used as nutrient to grow plants

3.8
culture solution
solution containing *liquid fertilizer* (3.7) supplied to hydroponic cultivation system

3.9
photosynthetic photon flux density
PPFD
number of photons per unit of time and area, which are contained in 400 nm – 700 nm of wave length needed for photosynthesis

4 Test method for growth promotion performance on lettuce

4.1 Conditions of test environment

4.1.1 Water supply. Sufficient water supply shall be provided to circulate the water in the piping of the hydroponic bed and the tank.

4.1.2 Air quality. There shall be no significantly abnormal values that would have impact on lettuce growth, compared with normal air composition.

4.1.3 Operation of growth promotion system. Continuous operation shall be available for the whole period of the test. The test method shall be applied to the growth promotion system and continuous operation shall be maintained for the whole period of the test. Each operation parameter related to stable fine bubble generation shall allow monitoring.

4.1.4 Performance of fine bubbles Each operation parameter related to stable fine bubble generation shall allow monitoring. Sizes and number concentrations of fine bubble water can be measured by the user own test or system supplier's data may be provided. However, when measurement is carried by the user, it is not necessary after mixing the liquid fertilizer.

4.1.5 Measuring systems. Measuring devices are required to monitor environmental parameters such as atmospheric temperature, carbon dioxide concentration, relative humidity, water temperature, dissolved oxygen, pH, electric conductivity and PPFD, as well as sample performance through measurement of the mass of stems and leaves.

4.2 Test system and related apparatus

The test system shall consist of the following (see Figure 1).

4.2.1 Configuration of test system. Two-line systems are used. One is for fine bubble water that is generated when the raw water is supplied to the fine bubble generating system and is supplied to the fine bubble section of the plant factory for promoting the growth of lettuce. In the fine bubble section, two or more sections can be used for the test. Meanwhile, another one is for the raw water without fine bubbles as a reference for the fine bubble section and is directly supplied to the control section of the plant factory. Figure 1 shows the basic layout of test line. Test apparatus is composed of fine bubble generating system, water tank, water supply system including pump, planting system and lettuce under growing stage, and has two planting lines. The planting system shall be installed in artificial light only, sunlight only or sunlight-artificial light type plant factory.

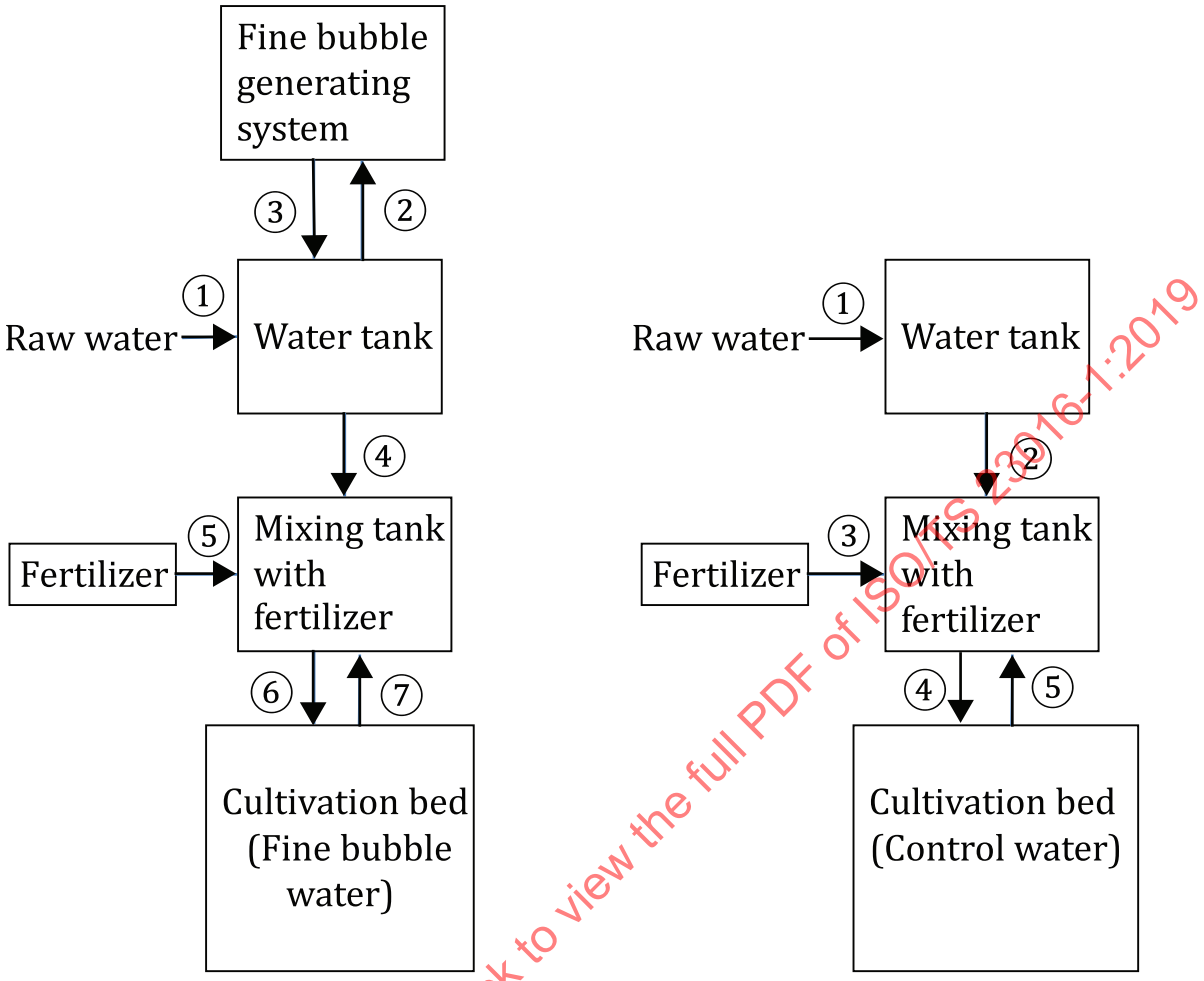
4.2.2 Fine bubble water line system. The fine bubble water mixed with liquid fertilizer in the liquid fertilizer mixing tank after the generation of fine bubbles in the bubble generation tank is supplied to the one (line for supplying fine bubble water) of two lines. Regarding liquid fertilizer, fine bubble water and liquid fertilizer are supplied by a fixed amount in the liquid fertilizer mixing tank. Alternatively, water and liquid fertilizer can be simultaneously supplied into a mixed tank, circulated through fine bubble generating system and returned to the mixed tank. The water tanks in Figure 1 can be combined. For fine bubble water, two or more sections can be used for tests.

4.2.3 Control water line system. Raw water and liquid fertilizer are supplied to the other line, by a fixed amount, to mix them after the raw water is supplied to the tank.

4.2.4 Tank. It is desirable that the inner walls of the tank are covered with materials with gas-barrier properties including glass to prevent the surface of the water in the tank from exposure to the air, and to ensure that the flow in the tank is in laminar flow conditions.

4.2.5 Water flow. The dimensions of the hydroponic bed, the volume of water including the hydroponic bed, tank and the piping as well as the water flow in the hydroponic bed, etc. shall be recorded in a test report.

4.2.6 Weighing scale. A calibrated weighing scale which can read to 0,1 g.



NOTE The numbers show the flow order of water, liquid fertilizer and mixture of these solutions.

Figure 1 — Configuration of the test system

4.3 Materials for test

For each test line with fine bubble water and control water, $(N + 2) \times 10$ stems and leaves of lettuces are used for the test. The harvesting date shall be between N weeks and $(N + 1)$ weeks from the sprouting.

4.4 Preparation for test

4.4.1 Confirmation of the working conditions of the fine bubble line system

Use raw water with fine bubble generating system that has been confirmed to generate fine bubbles of less than 100 µm diameter, preferably ultrafine bubbles of less than 1 µm diameter.

4.4.2 Hydroponic bed system

The hydroponic bed shall be installed in artificial light only, sunlight only or sunlight-artificial light type plant factory.

4.4.3 Operator(s) and inspector(s)

The production and shipping processes such as dissemination, transplant, harvest and shipment works and the test on growth promotion performance evaluation shall be carried out by trained staff to ensure consistent handling of all operations listed above.

4.4.4 Operation manual

Before performing the test, operation manuals shall be prepared with respect to dissemination, transplant, harvest, shipment works, growth promotion performance evaluation and other operations and remarks.

4.5 Preliminary test for confirmation of reproducibility

4.5.1 General

Before starting the test, a preliminary test shall be conducted to evaluate the reproducibility of the growth promotion process. Through the preliminary test, the harvesting date can be confirmed between N weeks and (N + 1) weeks from the sprouting. The test results are recorded as in 4.8 m).

4.5.2 Confirmation of reproducibility

In the preliminary test, lettuce is grown at two control sections without using fine bubble water and it should be confirmed whether the test results between both have any significant difference. It should be statistically examined by applying t-test based on the hypothesis that the results are identical with the analysis of variance (ANOVA). If there is no statistically significant difference, these sections are recognized showing a good reproducibility. With a good reproducibility, the proper test is conducted by changing one area of the two areas to fine bubble water.

4.5.3 Actions when there is a statistically significant difference

If there is a statistically significant difference, another combination of two lines should be retested or the combination with a smaller difference should be chosen.

4.6 Test procedure

4.6.1 Preparation of lettuce seedling

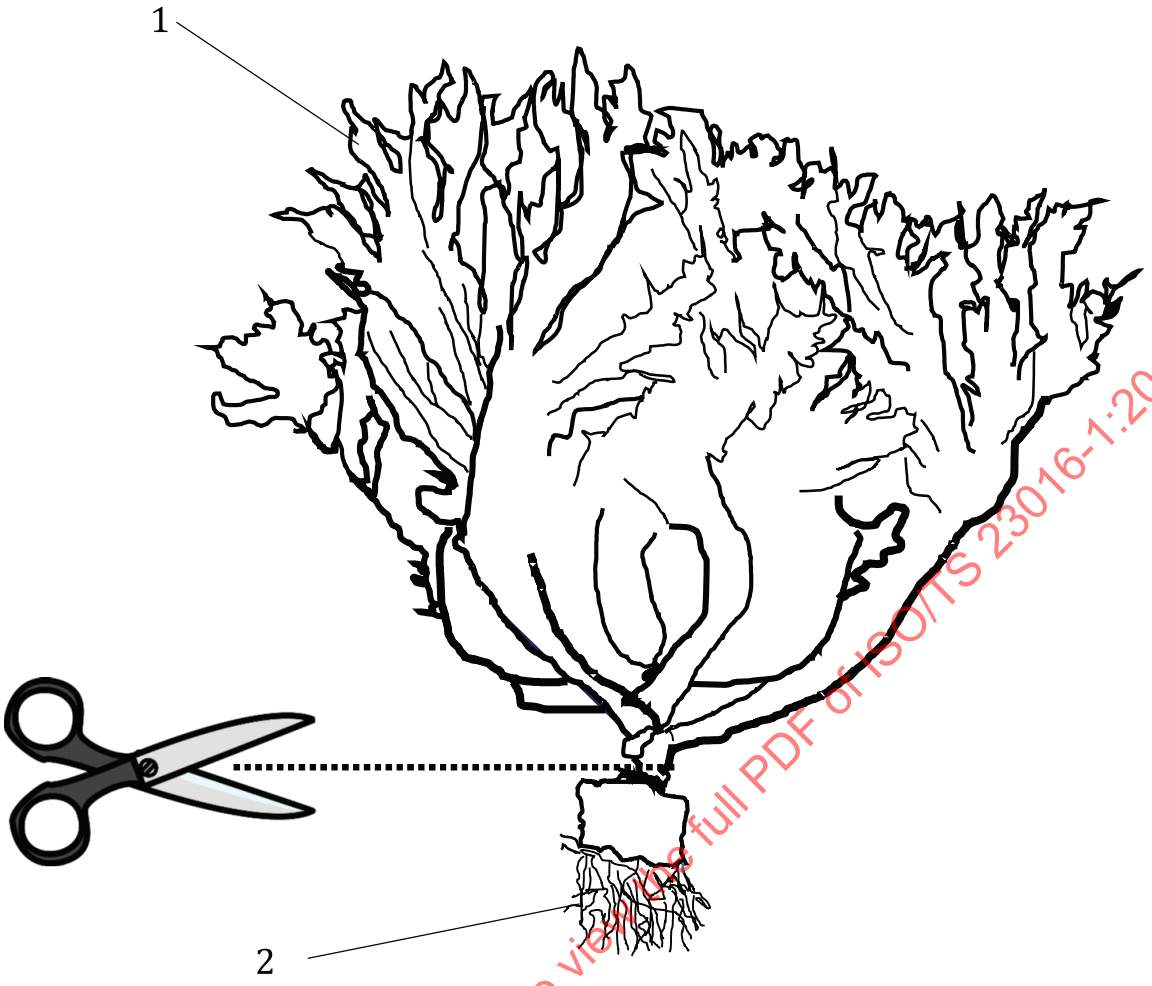
All specimens shall be uniformly grown under the same conditions.

4.6.2 Growth system

Lettuces shall be tested until (N + 1) weeks and the same test shall be implemented three times. Individual conditions using an artificial light only, sunlight only or sunlight-artificial light type plant factory shall be fixed.

4.6.3 Sampling

Ten lettuces shall be randomly picked up from the fine bubble section and the control section every week. The mass of stems and leaves being cut off as shown in Figure 2 shall be weighed within 30 min.



Key

1 part of stem and leaves

2 part of root

Figure 2 — Method of cutting between leaves and roots

4.6.4 Records

Record environmental parameters such as atmospheric temperature, relative humidity, water temperature, dissolved oxygen, pH, electric conductivity and carbon dioxide concentration and PPFD at a certain time during the period of the growth of lettuce. Liquid fertilizer composition is recorded before test after preparation.

4.6.5 Number of tests

The test shall be conducted in three replicates.

4.7 Calculation of degree of growth promotion

Measure weekly the mass of each lettuce sample prepared in accordance with 4.6.3 from the fine bubble section and the control section and calculate the average mass of each 10 stems and leaves obtained from each section. The degree of growth promotion performance (mass) is given by Formula (1):

$$\overline{m} = \left(\frac{F_N - C_N}{C_N} + \frac{F_{N+1} - C_{N+1}}{C_{N+1}} \right) / 2 \quad (1)$$

where

$\overline{m}$ is the growth promotion ratio;

F is the average mass of stems and leaves of lettuce in the fine bubble section;

C is the average mass of stems and leaves of lettuce in the control section;

N is the number of weeks when the harvesting date is intermediate between N weeks and $(N + 1)$ weeks from the sprouting.

An example of test results obtained for lettuce growth is shown in Annex A.

4.8 Test report

The test report shall contain the following information:

a) name and address of the test laboratory, test date, operator(s) and inspector(s);

b) the reference of this document, i.e. ISO/TS 23016-1;

c) specific number and model of the fine bubble generating system;

d) size and number concentration of fine bubble generating system (measured by the user or data provided by system suppliers);

e) system operation (e.g. whether the fine bubble generating process is continuous or batch system) and duration of storage of fine bubble water prior to mixing with liquid fertilizer;

f) weekly average masses and their standard deviation for both fine bubble and control sections (see A.1 as an example);

g) test environment conditions: dimensions of hydroponic bed, its water depth and water velocity;

h) type name of lettuce and age of seeds;

i) method of dissemination and growth, test term, testing with or without preliminary term;

j) time when distinctive incremental growth is observed in the test section using fine bubble water, e.g. 20 % incremental growth at the fifth week after commencement of test;

k) dosage of liquid fertilizer;

l) measurement figures of environmental parameters (see Annex B as an example);

m) information on test results reproducibility preferably including *t*-test in the preliminary test (test results should be treated subject to the agreement between the test laboratory and the user).

Annex A
(informative)

Example of test results for lettuce growth promotion performance

A.1 Example of recording format for measurements of test lettuce mass

An example of record table of average measurement values of test green leaf lettuce mass is shown in Table A.1.

Table A.1 — Example of record table for average measurement values of test lettuce mass

Week	First test average mass of 10 lettuces (g)		Second test average mass of 10 lettuces (g)		Third test average mass of 10 lettuces (g)	
	Fine bubble section F_{N1}	Control section C_{N1}	Fine bubble section F_{N2}	Control section C_{N2}	Fine bubble section F_{N3}	Control section C_{N3}
1	Disseminated		Disseminated		Disseminated	
2	Seedlings raised		Seedlings raised		Seedlings raised	
3	3,2 ± 0,21	3,3 ± 0,17	3,8 ± 0,25	4,0 ± 0,19	2,4 ± 0,24	3,1 ± 0,16
4	13,7 ± 0,51	13,1 ± 0,43	16,9 ± 0,58	15,2 ± 0,48	19,4 ± 0,65	16,2 ± 0,51
5	71,7 ± 4,49	58,5 ± 4,21	79,2 ± 4,72	65,0 ± 4,35	77,3 ± 4,80	59,2 ± 4,32
6	174,1 ± 9,98	152,0 ± 9,58	149,0 ± 9,28	123,6 ± 8,90	127,1 ± 9,02	113,5 ± 8,67
$\overline{m}$	0,19		0,21		0,21	

NOTE In the third week the seedlings were transplanted and the test started.

The following formulae are used for the calculation of average and standard deviation σ.

$$F_{N1} = \left(F_{N1(n=1)} + F_{N1(n=2)} + F_{N(n=3)} + \ldots + F_{N(n=10)}\right)/10 \tag{A.1}$$

$$C_{N1} = \left(C_{N1(n=1)} + C_{N1(n=2)} + C_{N(n=3)} + \ldots + C_{N(n=10)}\right)/10 \tag{A.2}$$

F_{N2}, C_{N2}, F_{N3} and C_{N3} are calculated in the same way.

$$\sigma\left(F_{N1}\right) = \left[\begin{array}{l}\left(F_{N1} - F_{N1(n=1)}\right)^2 + \left(F_{N1} - F_{N1(n=2)}\right)^2 + \\ \left(F_{N1} - F_{N1(n=3)}\right)^2 + \ldots + \left(F_{N1} - F_{N1(n=10)}\right)^2\end{array}\right]^{1/2} \tag{A.3}$$

$$\sigma\left(C_{N1}\right) = \left[\begin{array}{l}\left(C_{N1} - C_{N1(n=1)}\right)^2 + \left(C_{N1} - C_{N1(n=2)}\right)^2 + \\ \left(C_{N1} - C_{N1(n=3)}\right)^2 + \ldots + \left(C_{N1} - C_{N1(n=10)}\right)^2\end{array}\right]^{1/2} \tag{A.4}$$

$\sigma(F_{N2})$, $\sigma(C_{N2})$, $\sigma(F_{N3})$ and $\sigma(C_{N3})$ are calculated in the same way.

where

F, C and N are defined in 4.7;

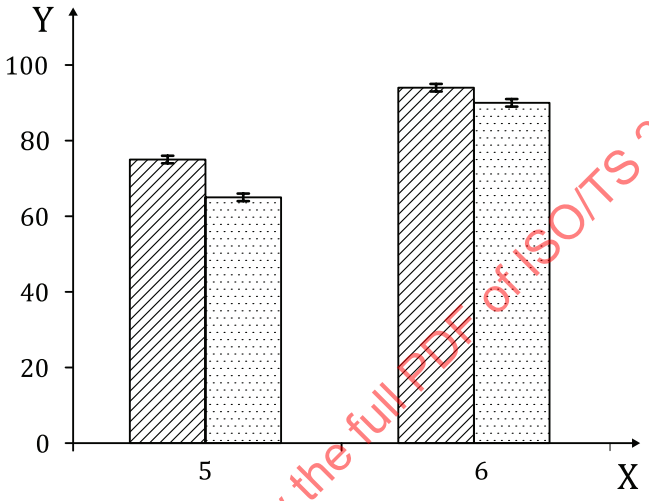
n is the number of lettuces whose stem and leaves are weighed;

$\overline{m}$ is the growth promotion ratio, with N = 5 being used in this case.

A.2 Graphic example of test results

The test data of green leaf lettuce and frilled lettuce are shown in Figure A.1 and Figure A.2.

The error bars on the bar graphs in the figures indicate a double value of the standard deviation.



Key

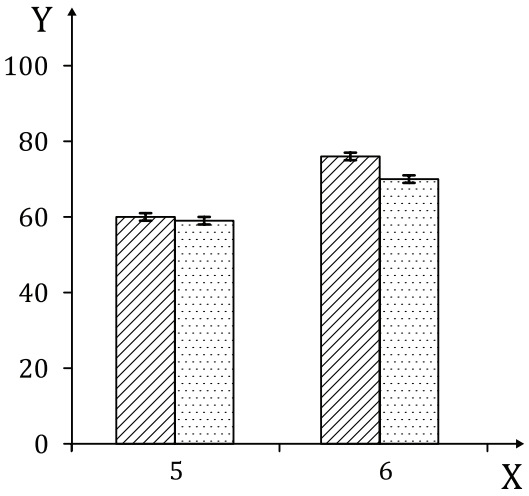
X duration of growth (weeks)

Y mass of stem and leaves (g)

▨ fine bubble water

⬚ control water

Figure A.1 — Mass of green leaf lettuce — Comparison of lettuce growth using fine bubble water and control water at week 5 and week 6



Key

X duration of growth (weeks)

Y mass of stem and leaves (g)

▨ fine bubble water

▩ control water

Figure A.2 — Mass of frilled lettuce — Comparison of lettuce growth using fine bubble water and control water at week 5 and week 6