Properties of the water containing nanobubbles as a new technology of the acceleration of physiological activity

Shu Liu[1], Masatoshi Enari[1], Yoshinori Kawagoe[2], Yoshio Makino[1], Seiichi Oshita[1]*

[1] *Graduate School of Agricultural & Life Sciences, The University of Tokyo Yayoi 1-1-1, Bunkyo-ku, Tokyo 113-8657, Japan*
[2] *College of Bioresource Sciences, Nihon University, Kameino 1866, Fujisawa, Kanagawa 252-0880, Japan*
**Corresponding author. E-mail: aoshita@mail.ecc.u-tokyo.ac.jp*

Abstract

The mechanism of nanobubbles' (NBs) physiological activity promotion was investigated with the Proton Nuclear Magnetic Resonance (NMR) relaxation time measurements. According to the experiment results, the number of NBs had a positive correlation with the spin-spin relaxation time (T_2) value of the water, which meant introducing NBs could increase the mobility of water in bulk. These results suggested that the NBs in water could influence the physical properties of water and that it could contribute to one of the explanations for the mechanism of NBs' promotion effect on physiological activity of living organisms. The hydroponic experiment showed that the NBs themselves could greatly promote the growth of barley and NBs technology was feasible to be used in hydroponic cultivation of vegetables as a new technology in agriculture applications.

Key words: Proton spin-spin relaxation time T_2, water mobility, degassing, hydroponic experiment, root system.

1. Introduction

In recent years, Micro and nanobubbles (MNBs) technology has attracted people's great concern in the biological field. Many researchers reported MNBs' effect on physiological activity through laboratory experiments. It has been reported that the water containing MNBs can accelerate the growth of plants and shellfish and it can also be used in aerobic cultivation of yeast. Kurata et al. (2008) who applied oxygen micro-bubbles in osteoblastic cell culture, proved higher alkaline phosphatase activity, which was related to the higher osteoblastic cell activity. Park and Kurata (2009) found that the fresh weights of the microbubble-treated lettuces were 2.1 times heavier than those of the macrobubble-treated lettuces under the similar dissolved oxygen concentration. Ushikubo et al. (2008) showed when the barley coleoptile cells floated in water after the generation of oxygen MNBs, cytoplasmic streaming rates inside the cells were accelerated.

At the same time, the promotion effects of MNBs on physiological activities were also approved in the field experiments. The MNBs technology was applied in Tawarayama hot spring at Nagato, Yamaguchi, and it was found that compared to the normal hot spring water, the skin surface temperature in the MNBs hot spring water increased 0.6 ^{o}C (Onari, H., 2007). In Tokuyama, MNBs technology was used to enhance the growth of water hyacinth (*Eichhornia crassipes (Martius) Solms*-Laubach) for two years. Compared to the water without MNBs, the root length and the root weight of water hyacinth cultivated with MNBs water were increased 3.9 and 4.8 times, respectively (Nakata, A., 2004).

The effectiveness above cannot be explained only by the increase of the dissolved oxygen, and the MNBs themselves should play an important role in the physicology activity in cells. Up to now, there have been many unclear questions concerning the nanobubbles (NBs). According to the classical thermodynamic theory, the lifetime of NBs was less than 0.1μs (100 nm in radius) (Ljunggren, S. and Eriksson, J.C., 1997). Though many researchers have reported their existence in water through experiments (Ushikubo, F.Y. et al. 2010; Uchida, T. et al., 2011), the details of NBs' formation and stability are still poorly understood (Brenner, M.P. and Lohse, D., 2008). Thus, the basic research on physicochemical properties of the water containing NBs becomes very important.

Just recently, it was found that the spin-lattice relaxation times (T_1) and spin-spin relaxation times (T_2) of the germinated seeds dipped in the water containing NBs were both significantly higher than those in the distilled water (Liu, S. et al., 2012). Moreover, Nagarajan's research showed that T_1 and T_2 in leaves were directly related to water activity of the cell water, which in turn was related to availability of T_1 and T_2 as a probe for metabolic activities.

In view of this, the study on the effect of NBs on physical properties of water in bulk through the spin-spin relaxation times T_2 in both the water with and without NBs may be an appropriate and feasible way to figure out the reason for NBs' physicology activity promotion. At the same time, the hydroponic experiments were designed in the solution with and without NBs under the same DO concentration to verify the role of NBs themslves in the physiology activity of plants and the feasibility of MNBs technology in hydroponic cultivation of vegetables.

2. Material and methods

2.1 Properities of water with and without NBs

Control water

The desired control water has low DO concentration and is free of bubbles. Bubbling method was used so that the DO in the ultrapure water (Direct-Q, Nihon Millipore Ltd., Japan) could be removed. Dissolved gasses in the ultra-pure water of 2l were purged by introducing nitrogen gas directly through a tube with 4 mm diameter inside. The dissolved oxygen concentration (DO) of this control water was below 0.15mgl^{-1} and few bubbles were observed through a laser scattering image system (Zeecom, Microtech Co. Ltd., Japan).

Water containing NBs

Control water was placed into an Erlenmeyer flask. The gas (N_2, purity 99.99995 %) was introduced into the water through a Micro-bubble Generator (OM4-GP-040, Aura Tec Co. Ltd., Japan) for 1 hour at the constant temperature of 20°C to obtain the "water containing NBs". The difference of DO concentration between the control water and the water containing NBs was less than 0.05mgl^{-1}.

Sample for NMR measurement

For the NMR measurements, the volume of water samples is normally 0.4ml. In order to eliminate the paramagnetic effect of dissolved oxygen, the NMR tubes were filled with water and then were sealed. All the tubes were stored in an incubator at 20^{o}C.

Degassing

In order to remove the bubbles in the water, a vacuum pump (5L/min, 50Hz) was used to degas the water samples. The pressure for the degassing process was about 0.02MPa. After the water was degassed, the head space of the NMR tubes was filled with nitrogen to keep the water sample under atmospheric pressure. The degassing was done in a water bath of 30^{o}C.

NMR measurement

Proton Spin-lattice relaxation time (T_1) and spin-spin relaxation time (T_2) were measured by a pulsed spectrometer (JNM-MU25A, JEOL, Japan) at 25MHz frequency and at the constant temperature (20^{o}C). The pulse sequences used for T_1 and T_2 were saturation recovery and Carr-Purcell-Meiboom-Gill (CPMG), respectively. Five replications of each sample were collected in NMR tubes with the diameter of 10mm outside, and then were sealed.

2.2 Hydroponic experiments

Seed material

Seeds of barley (*Hordeum vulgare* L.) which were harvested in 2011, stored under controlled storage (4^{o}C) were obtained from University of Ehime, Japan. These seeds were screened with a magnifying lens and only big seeds without visible defects were selected.

Control water

Distilled water was used as the control water. The DO concentration of this distilled water was about 9 mg/L, and the pH value was about 6-7.

Water containing NBs

The mixed gas of nitrogen (purity 99.99995%) and air (CO_2<1ppm, THC<1ppm) was introduced into the distilled water through the micro-bubble generator for 1 hour at the constant temperature of 20°C to obtain the "water containing NBs". The dissolved oxygen (DO) concentration of the water containing NBs was adjusted to be the same as that of the distilled water through a mixed gas flow regulator (Log MIX-D100A-0050 and Log MIX-D100A-0052).

Hydroponic culture conditions

After the germination, the obvious germinated seeds in both the water containing NBs and the distilled water were selected. Two culture containers were prepared for the seeds germinated in the water containing NBs and two for the seeds germinated in the distilled water. A sponge with 7×4 holes was fixed in each culture container. In every other hole placed a seed, and in each container sow 14 seeds. The culture containers were all placed in an incubator. The temperature was maintained at 20°C, and a light source provided light for 11 hours and darkness for 13 hours for the plant growth every day. High concentration of KNOP nutrient solution was diluted 20 times with the water containing NBs and with the distilled water respectively, and then was applied respectively to the germinated seeds in the water containing NBs and in the distilled water.

The water in the container was exchanged every day. On the final day of cultivation (12 days and 14 days), the barley plants were detached from the sponge. And the leaf length, the root length and number, and the dry weight and wet weight of the total plants were recorded. Then the total plants were placed in a dry oven at 105°C for 24 hours to get the dry weight of each plant. Statistical analyses were performed with the EXCEL 2007 software.

3. Results and discussion

3.1 The influence of NBs on the physicochemical properties of water

Change of T_2 values of the control water and the water containing NBs with water storage time

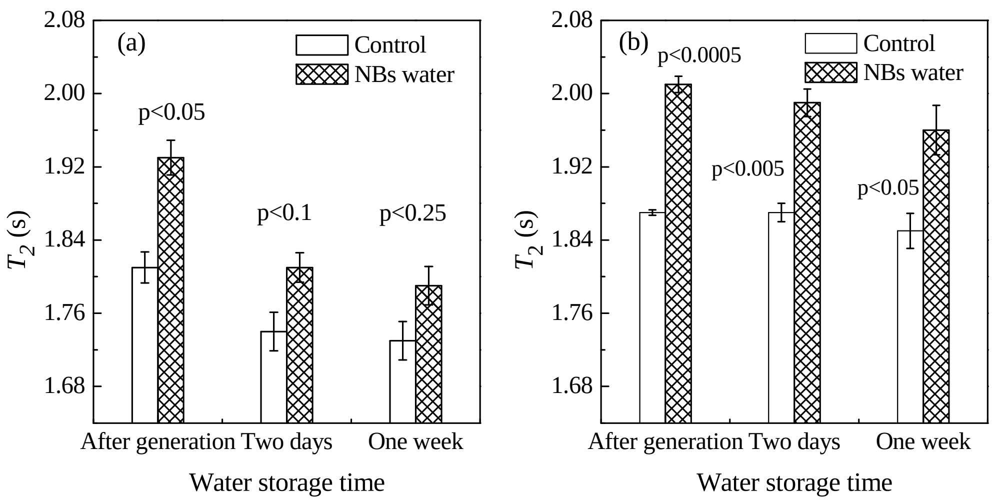


FIGURE 1: Time-dependent change of T_2 values of the control water and the water containing NBs at 20°C (a: Water sample of 0.4ml with low DO concentration in the NMR tube under air atmosphere; b: Water samples filled in pressure-tight tubes with no head space above the water)

The water containing NBs displayed statistically longer T_2 values than those of the control water as shown in Figure 1a. The sample temperature was 20°C. The pH values for the control water and the water containing NBs were 6.31 and 7.06 respectively. T_2 values of both the control and the water containing NBs shortened after 2 days, and their difference in T_2 values observed on the day of generation became unclear with time. The reason for both the shortening of T_2 values and the decrease of the difference in T_2 was estimated as the oxygen (a paramagnetic molecule) in the head space of NMR tube dissolved into water, resulted in elevated DO in the sample water, and consequently reduced T_2. At the same time, the bubble size distributions were measured with Dynamic Light Scattering method (NanoSight-LM10, Quantum Design Japan, Inc.). The bubble number density became less when storage time was longer (data not shown). Therefore, the effect of NBs on T_2 was partly masked by the paramagnetic effect of oxygen at the observation after 2 days and more.

The presence of oxygen, a paramagnetic molecule, would shorten the relaxation time. Aiming to eliminate the paramagnetic effect of oxygen in the head space, we filled both the control water and the water containing NBs in the pressure-tight tubes to ensure that there was no gas in them. As can be seen in Figure 1b the water containing NBs displayed statistically longer T_2 values than those of the control water. The sample temperature was 20^{o}C. The pH values for the control water and the water containing NBs were 7.91 and 8.01 respectively. T_2 values of both the control water and the water containing NBs still didn't change much after a week passed by, and their difference in T_2 values was still obvious.

Change of T_2 values of the control water and the water containing NBs after degassing process

The results from the experiments above showed that introducing NBs did increase the T_2 value of water. In order to clarify the relationship between the T_2 value of the water and that of the water containing NBs, degassing was used to remove the NBs in the water sample. If the NBs were the only factor which caused the increase in T_2 of the water, then after the degassing, the T_2 of the water sample should decrease and restore to the original T_2 value.

After the T_2 measurement, both of the water containing NBs and the control water were degassed under the pressure of 0.02MPa. Then, T_2 was measured again in order to examine the disappearance of NBs due to degassing. As can be seen in Figure 2a, the T_2 values of one among five samples for the water containing NBs with degassing time decreased from 2.03 seconds to 1.92 seconds.

After the decreasing tendency of T_2 value had been confirmed with degassing time, the other 9 water samples (Five for the control and four for the water containing NBs) were also degassed for 45 minutes at 30^{o}C. As can be seen in Figure 2b, the T_2 values for the water containing NBs obviously decreased after the degassing. Moreover, after the degassing, the T_2 of the water containing NBs was still statistically longer than that of the control water.

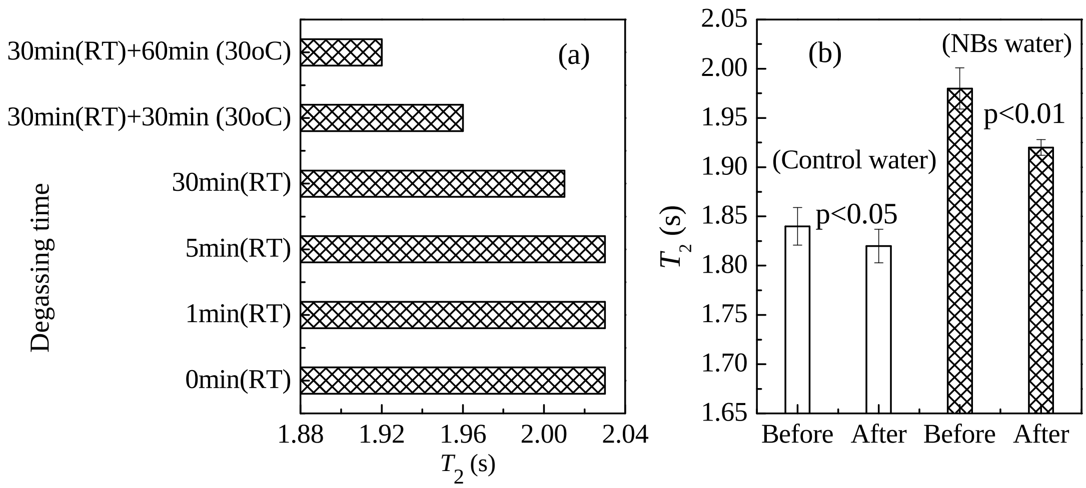


FIGURE 2: T_2 of the control water and the water containing NBs before and after degassing measured at 20^{o}C (a: change in T_2 of one of 5 water samples as the water containing NBs during degassing; b: change in mean value of T_2 of the control water and the water containing NBs before and after degassing; The degassing pressure is 0.02MPa; RT: room temperature)

For the water containing NBs, after degassing, some large bubbles formed from the coalescence of several NBs. Henry's law suggests that exposing a water droplet to an external pressure lower than atmospheric pressure can reduce the solubility of air and hence results in local super saturation of dissolved gas in the droplet. As the bubbles increase in size, they may engulf other NBs nearby, even if they have most likely the same surface charge. Once they are large enough, the bubbles may detach from the bulk water because of buoyancy. After the degassing, some large bubbles were actually seen with our naked eyes.

Each large bubble was formed from a large number of NBs. For the control water, after the degassing, the T_2 values also decreased. However, the decrease rate was much lower than that of the water containing NBs. Therefore, we concluded that the control water might also contain a small number of NBs which were much fewer than those in the water containing NBs.
Based on the results above, we concluded that the number of NBs had apparently positive correlation with the T_2 value of the water, indicating that NBs could increase the mobility of the water molecules in bulk. These results suggested that the NBs in water could influence the physicochemical properties of water and that it could contribute to one of the explanations for the mechanism of NBs' promotion effect on physiological activity of living organisms.

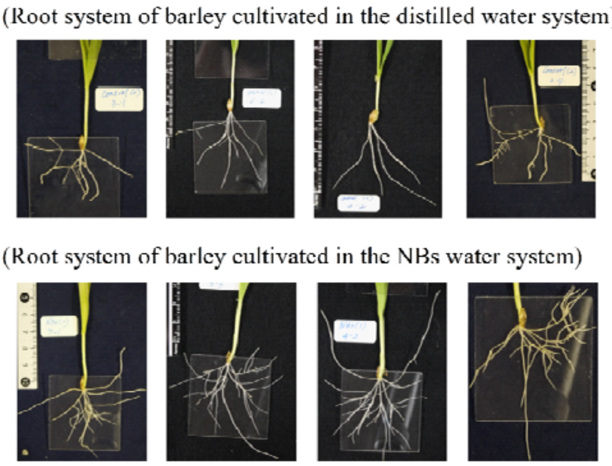
3.2 The feasibility of NBs technology in hydroponic cultivation of barley

NBs effect on the promotion of physiological was then applied in the hydroponic experiments to verify the feasibility of this technology in hydroponic cultivation of vegetables.

TABLE 1. Root length, leaf length, dry weight, and wet weight of barley grown for about 2 weeks (1# for 12 days, 2# for 14 days) in hydroponic culture system with and without NBs generated in nutrient solution.

Experiment	Solution	Total root length/mm	Total leaf length/mm	Dry weight of total barley/g	Wet weight of total barley/g
1#	NBs solution	413.33±24.83	201.76±5.01	0.029±0.001	0.301±0.011
	Control solution	293.62±24.77	164.44±9.95	0.023±0.002	0.240±0.020
2#	NBs solution	423.00±37.04	204.20±8.29	0.031±0.001	0.316±0.015
	Control solution	324.96±23.93	180.55±6.89	0.029±0.001	0.303±0.014

As can be seen in TABLE 1, two parallel experiments showed that after 12 days and 14 days cultivation, the total root lengths of barley cultivated in the water containing NBs were respectively 1.41 and 1.32 times longer than those cultivated with distilled water in our two experiments. Moreover, both the outer leaf and the second leaf of barley cultivated in the water containing NBs were significantly longer than those with distilled water ($p<0.05$). However, the wet weights of the barley in the water containing NBs system and in the distilled water system were not significantly different and the dry weights were the same.



PICTURE 1 The photo for the root systems of barleys cultivated in two kinds of water (We selected the average level of barleys in two culture systems)

As showed in the PICTURE 1, the seeds of plants with more developed root system were shriveled because their nutrition was offered to the plants. On the contrary, the seeds of plants with less developed root system were more saturated. This might be the reason why the weights were not significantly different between the barley in the water containing NBs system and the distilled water system.

On the other hand, from PICTURE 1, it can be concluded that barley cultivated in the water containing NBs had more developed root system than those cultivated in the distilled water. The initiation and development of roots can be critical to the growth and productivity of crop plants (Gifford, R.M. and Evans, L.T., 1981). Roots are recognized as metabolic sinks that influence the partitioning of photosynthetically fixed carbon. Formation of adventitious roots has been associated with increased carbon fixation rates (Robbins, N.S., 1988). These results indicated that NBs technology could be adapted effectively in hydroponic culture system.

Conclusions

According to the NMR results, the number of NBs had a positive correlation with the T_2 value of the water, indicating that NBs could increase the mobility of the water molecules in bulk. These results suggested that the NBs in water could influence the physicochemical properties of water and that it could contribute to one of the explanations for the mechanism of NBs' promotion effect on physiological activity of living organisms. The hydroponic experiment showed that the NBs could greatly promote the growth of barley and NMBs technology was feasible to be used in hydroponic cultivation of vegetables as a new technology in agriculture applications.

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