NANO-BUBBLES' EFFECTS ON THE PHYSICOCHEMICAL PROPERTIES OF WATER – THE BASIS OF PECULIAR PROPERTIES OF WATER CONTAINING NANO-BUBBLES –

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Abstract

Proton NMR relaxation times, T_1 and T_2 of the water containing nano-bubbles (NBs) were measured to compare with the control water. The water containing NBs displayed statistically longer T_2 values than those of the control water. The increase in T_2 by the generation of NBs meant that the mobility of the water molecules increased and consequently a longer period was required to reach equilibrium of the spin-spin relaxation process. These observations indicated that the NBs in water could influence the physicochemical properties of water and that it could be used to verify the stability of nano-bubbles in the water.

Keywords

proton relaxation time T_1, T_2, water mobility, stability of nano-bubbles

Introduction

While micro- and nano-bubble (MNB) technology has widely been applied in various fields, people are showing more and more interest and attention to the fundamental research of the MNB mechanism. As much as to say that researchers are not only satisfied with their research on the application of the MNB but they also want to do the further research on the reasons why the MNB have such effects on their applications. Compared to the normal bubbles, MNB have mainly the following characteristics: smaller bubble sizes which cause the lower buoyancies; either negative or positive zeta potentials; larger specific areas; higher internal pressures which cause the increase of dissolution of gas in the liquid; free-radicals generation with the collapse of micro-bubbles under the water; the ability of reducing friction.

Due to the characteristics above, MNB technologies have been applied in many research fields such as medical, medicine, environmental engineering, horticulture and agricultural, food science and technology, fluidic physics and mineral processes.

Recently, more attention has been focused on application of MNB technology in biological processes. It has been reported that the water containing MNB can accelerate the growth of plants and shellfish and it can also be used in aerobic cultivation of yeast. The air micro-bubble supply in the cultivation of oysters *(Heterocapsa circularisquama)* results in a better quality product in terms of size and in taste (Onari, 2001). Kurata et al. (2007) 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 lettuce were 2.1 times heavier than those of the macrobubble-treated lettuce under the similar dissolved oxygen concentration. Ushikubo et al. (2008) showed when the barley coleoptile cells floated in water after the generation of oxygen micro- and nano-bubbles, cytoplasmic streaming rates inside the cells were accelerated. In addition, Nakao et al. (2008) proved the enhancement of micro-bubble water on seed germination. At present, the explanation has not theoretically been given for these new scientific findings. At this point, the applied studies on MNB precede its fundamentals and some fundamental aspects of the water containing MNB still remain unclear. Thus, the basic research on physicochemical properties of the water containing MNB becomes very important.

Up to now, many researchers have put forward their opinions on physicochemical properties of the water containing MNB through experiments or models. According to Ohgaki et al. (2010), the water molecules may form shells of hard hydrogen-bonded icelike structures around the MNB that may reduce the diffusivity of gases through the interfacial film. Craig (2010) showed that the increase in the radius of curvature associated with the nanoscopic contact angle resulted in a much reduced internal pressure and contributed to the stability of MNB. Jin et al. (2007) found that the coverage of small amphiphilic organic molecules at the gas/water interface greatly reduced the pressure inside MNB. Hampton (2010) also says that a reduction on surface tension of vapor-liquid interface reduced the Laplace pressure and increased MNB stability. But none of the achievements above can explain the mechanism of the acceleration of metabolism.

Nuclear Magnetic Resonance (NMR) relaxation can detect weak molecular interactions such as hydrogen bonding, molecular mobility and steric effect (Balci, 2005). As a result, it is widely used to study the mobility and diffusion of water molecules in agriculture and food field. In view of this, we expect that NMR technology will be helpful to the research on the characteristics of the NBs in water. In this study, spin-lattice and spin-spin relaxation times (T_1 and T_2) were measured both in control water and the water containing NBs aiming to obtain the effect of NBs on physicochemical properties of water.

Material and methrods

Ultrapure water

Ultrapure water was obtained using a water purification system (Direct-Q, Nihon Millipore Ltd., Japan), which was equipped with a reverse osmosis cartridge and modules of ion-exchange and activated carbon. According to the result from Nanoparticle tracking analysis technology (Z-NTA, Quantum Design Japan, Inc.), the ultrapure water didn't contain any particles and bubbles. The ultrapure water naturally contained about 6mg/l dissolved oxygen.

Control water

The desired control water has low DO concentration and is free of bubbles. In order to remove the DO in the ultrapure water, bubbling method was used. 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 (N2, 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 measurement, the volume of water samples is normally 0.4ml. In order to eliminate the paramagnetic effect of dissolved oxygen, two methods were used in the research. For one method, the NMR tubes were filled with water and then were sealed. In the case of 0.4ml water placed in the tubes, the air was replaced by nitrogen. All the tubes were stored in an incubator at 20°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°C, 24°C and 30°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 pressure-tight tubes with the diameter of 8.5mm inside, and then sealed.

Bubble size and distribution

The NBs' size distribution measurements were performed using Nanoparticle tracking analysis technology. Based on a laser-illuminated optical microscope, NBs were seen as light-scattering centers moving under Brownian motion. After the NBs generation, water was stored in 15 BOD bottles in incubator at 20°C. Then the bubble size distribution was measured with different storage time.

DO concentration

The dissolved oxygen (DO) concentration was measured both in control and the water containing NBs at 20°C, using a DO meter (SG6, Mettler Toledo GmbH, Switzerland).

Statistical analysis

The statistical tests were performed with the EXCEL software 2003. Paired sample t-test was used to compare the difference between the control water and the water containing NBs with a significance level (p-value) set from 0.0005 to 0.25.

Results and disscussions

Water sample of 0.4ml in pressure-tight tubes

The water containing NBs displayed statistically longer T_2 values than those of the control water as shown in Fig. 1. 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 control and the water containing NBs shortened 2 days later, and their difference in T_2 values observed on the day of generation became unclear with time as shown in Fig. 1. Under a normal condition, the significance level higher than 0.05 is of little meaning. In order to compare the difference between the control water and the water containing NBs with different storage time, test levels of p values higher than 0.05 (0.1 and 0.25) were also involved. The reason for both the shortening and the decrease of the difference in T_2 was estimated as the oxygen in the head space of NMR tube dissolved into water and elevated DO in the sample water caused T_2 shorter. At the same time, the bubble number density would become smaller with time. Therefore, the effect of NB on T_2 was partly masked by the paramagnetic effect of oxygen at the observation after 2 days and more. The mean T_1 value on the day of generation of both the water containing NBs and the control water was 2.48s. T_2 is more sensitive comparing with the T_1, and meanwhile there will be a few contaminants of foreign materials in the control water. It may be the reason why the difference between the control water and the water containing NBs could only be seen from T_2 values.

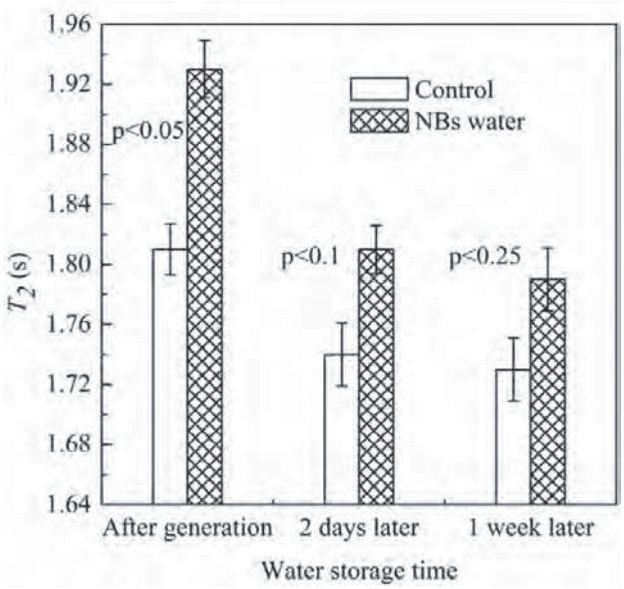

Figure 1. Change of T_2 values of control water and the water containing NBs with water storage time

The decrease of bubble number density with storage time was supported by the results shown in Fig. 2b in which both the total bubble number and the main bubbles size decreased with storage time. The main bubble size observed was divided in two sizes,

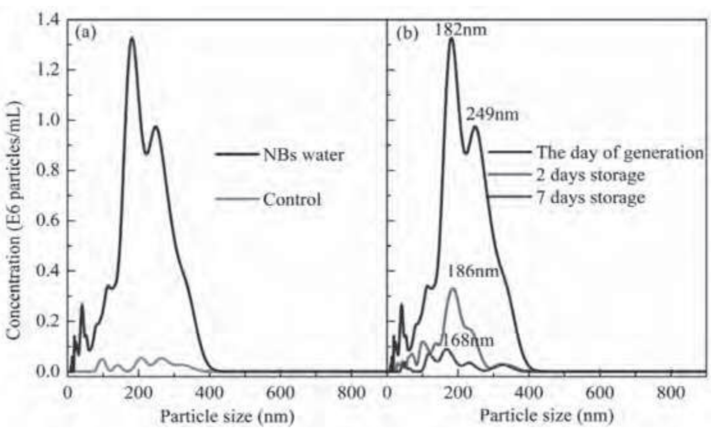

Figure 2. Bubble size distributions with time of control water and the water containing NBs. a shows the comparison of bubble size distribution of control water and the water containing NBs. b shows the change of bubble size distributions in the water containing NBs with different storage time

182nm and 249 nm on the day of generation, two days later 186nm, and seven days later 168nm. The shrinking tendency of the bubble sizes was shown clearly.

The total bubble number density of the water containing NBs was 1.97 $\times 10^8$ particles per milliliter on the day of generation. For the control water, the total bubble number density was about 0.09 $\times 10^8$ particles per milliliter, which was less than 1/20 of the water containing NBs (Fig. 2a). The measurement was done at room temperature, and the temperatures for the water containing NBs and the control water were 29.8°C and 28.7°C, respectively.

Water samples filled in pressure-tight tubes

The presence of oxygen, a paramagnetic molecule, would make the relaxation time shorter. Aiming to eliminate the paramagnetic effect of oxygen in the head space, both the control water and the water containing NBs were filled in the pressure-tight tubes to ensure that there was no gas in them. Fig. 3. showed results done at three different temperatures, 20°C, 24°C and 30°C. The mean T_2 values of the water containing NBs were all longer than those of control water. Moreover, the increase of sample temperature would enhance the difference in T_2 between the water containing NBs and the control water.

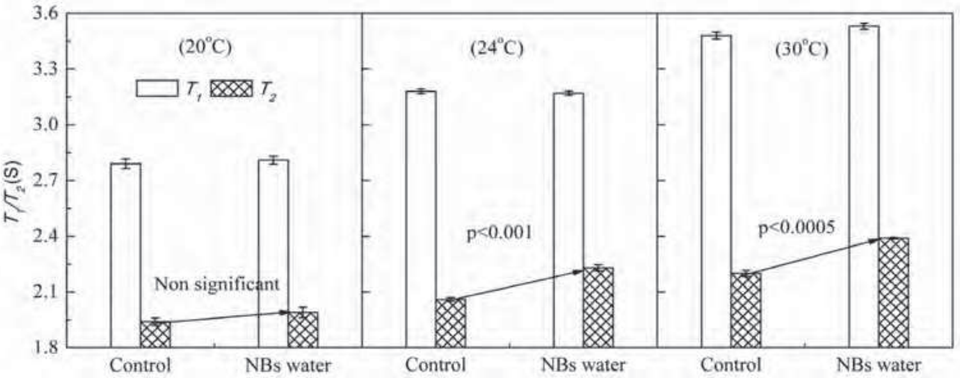

Figure 3. T_1 and T_2 values of control water and the water containing NBs under different temperatures (DO concentrations of both control water and the water containing NBs were lower than 0.15mg/l, their difference below 0.05mg/l)

In order to approve further effect of high temperature, both the T_1 and T_2 values were measured using the same set of samples under two different temperatures (24°C and 30°C). Sample set was the same as that used in Fig.3. at 24°C, and the measurement was done two days later. Fig. 4. shows more significant difference between the water containing NBs and the control water under the higher temperature.

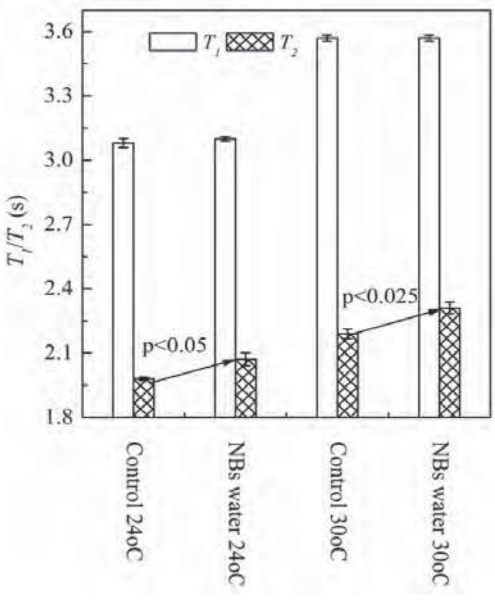

Figure 4. T_1 and T_2 values of control water and the water containing NBs under different temperatures after two days generation

Besides, the Zeta potential values of the water containing NBs were also measured. Two repeated experimental results showed that the Zeta potential of the water containing NBs was negative with the mean value of -32.26mV at pH 7.28 and -38.84mV at pH 7.55.

From the experiments above, the difference of T_1 values between the control water and the water containing NBs still couldn't be detected.

Water sample of 0.4ml in the NMR tube under nitrogen atmosphere

Replacing the air in the tube by nitrogen was another method to prevent the head space oxygen to dissolve into the water. Fig. 5. shows results under different measurement temperatures (20°C and 30°C). For the same results as above, the mean T_2 values of the water containing NBs were all longer than those of the control water and the increase of sample temperature would enhance the difference in T_2 between the water containing NBs and the control water. There is also no significant difference of T_1 values between the two kinds of water.

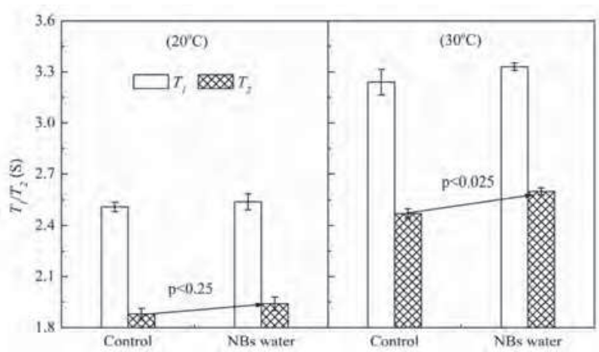

Figure 5. T_1 and T_2 values of control water and the water containing NBs under different temperatures, the head space of which was replaced by nitrogen

Discussion about the influence of NBs on the physicochemical properties of water

According to Ohgaki et al. (2010), the water molecules may form shells of hard hydrogen-bonded icelike structures around the MNB, and the higher surface tension (twice the normal value) arising from the presence of the hard interface helps to maintain a kinetic balance against the high internal pressure. On the contrary, Himuro (2007) found that shrinking of MNB greatly decreased the number of hydrogen bonded water molecules causing smaller surface tension. MNB contributed to weakening the hydrogen bonded network. We can't say which opinion is right, but the results from multiple parallel experiments showed that introducing NBs did increase the T_2 value of water.

One of our explanations is that the mobility of the water molecules increased and consequently a longer period was required to reach equilibrium of the spin-spin relaxation process. Takahashi (2005), who measured zeta potential in different solutions and distilled water with NBs, reported that bubbles were negatively charged, and OH- and H+ ions should play an important role in electrical charge. Negative-charged bubbles could adsorb hydrogen ions on their surface which made Coulomb repulsion forces compensate surface tension forces (Chaplin, 2007).The presence of ions, therefore, could modify the hydrogen bonding network in water. The hydrogen bond around the bubble surface may enhance the viscosity of water which was near the interface of bubbles and cause to decrease the mobility of a limited part of water molecules. But for the rest of the larger quantity of water, the NBs contributed to weakening the hydrogen bond network of the water (Fig. 6.). The bubbles' forming, shrinking and disappearing would affect the total hydrogen bond network and the structure of water as well. The disturbance of hydrogen-bond would accelerate the mobility of water molecules. The higher temperature might have destructing effect on hydrogen-bonds. And at the same time it might enhance the moving rates of both water molecules and NBs, which increased the chances of their collision.

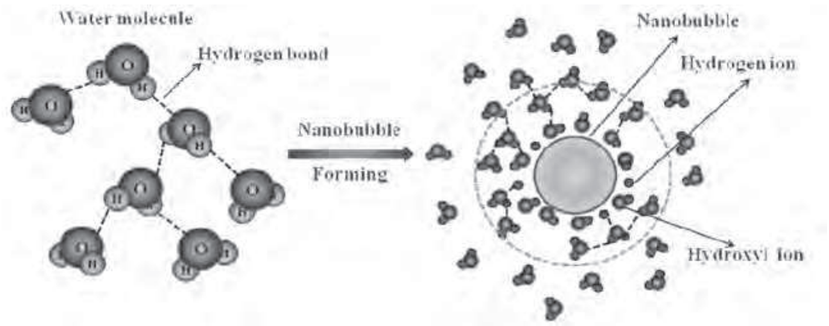

Figure 6. Nanobubbles effect on the hydrogen bond network and the structure of water. In the red circle were the water molecules near the interface of bubbles which formed hydrogen-bonded shell. Outside the red circle were the free water molecules whose hydrogen bond network was weakened by NBs.

The other explanation was that the magnetic field radiation might affect the gas-liquid interface leading to the destabilization of bubbles mostly by disturbing the ionic balance between the shell formed with water molecules of adsorbed negative ions and counterions. (Vallee et al., 2005).

No single theory can explain NBs' effect on physicochemical properties of water, and is more than likely due to a number of mechanisms, depending on the conditions of the system.

Water activity was the important factor that could accelerate the growth of bacteria and enzymatic reaction. The fundamental data showed here could contribute to explain the mechanism of the effect on the physiologic activity.

Conclusions

The water containing NBs displayed statistically longer T_2 values than the control water indicating that the mobility of the water molecules increased and consequently a longer period was required to reach equilibrium of the spin-spin relaxation process. Moreover, increasing the sample temperature would enhance the difference between them. From these findings, it was considered that the NBs in water could influence the physicochemical properties of water and that it could be used to verify the stability of NBs in the water.

Acknowledgements

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