Sterilization Effects of Ozone Fine (Micro/Nano) Bubble Water

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Abstract—Effects of sterilization of fine (micro/nano) bubble (FB) water of ozone gas were studied in relating to treatment times. In the applications of FB water, since hydroxyl radicals (OH•) were observed to occur in the water, it is expected that ozone FB water can have strong and long-duration sterilization effects, which is a very important function, in particular, for food safety applications. In order to verify the effects, more comprehensive experiments were made to clarify for FB water of ozone gas. The results showed that fresh ozone fine bubbles (O₃FB) water had the most effective inhibition of the growth of Escherichia coli when used right after 60 min generation. In the three days-storage O₃FB water, no sterilization effect was observed. The results of this study suggest that the application of fresh O₃FB water may be useful for sterilization process in the food industry.

Keywords—Fine bubble, ozone, sterilization

I. INTRODUCTION

Fine bubbles (FB) or micro/nanobubbles are often defined as tiny bubbles with diameters less than 100 μm. One of the most remarkable properties of FB is their tendency to decrease in size and subsequently to collapse under water. In contrast, the ordinary macrobubbles rise rapidly and burst at the surface of the water [1-4].

FB technology is an emerging and innovative technology applicable to versatile applications in many fields such as agricultural fields, medical fields, and food industry. Previous researches demonstrated that the application of microbubbles in deep-flow technique hydroponics culture system could significantly promote the growth of lettuce [5]. Moreover, reports on disinfection of wastewater using ozone microbubbles have discussed their strong disinfectant functions [6, 7]. Furthermore, some researchers indicated that nanobubble water possessed potent bactericidal activity against periodontopathic bacteria [8].

Among them, applications to food safety have drawn great attention in the food industries in Thailand, because of its outstanding capability vastly different from conventional methods. One of well-known practical applications of FB is supplying them to the culture of oysters, scallops and other marine products, and the growth rate has been proved to be greatly improved [9]. Among various FB water, the ozone FB water has strong oxidative activity and can be applied to various water treatment processes including sterilization [10, 11]. In addition, some researchers reported that microbubbles could accelerate the formation of the hydroxyl radicals during ozonation processes [6, 7, 12]. Previous study on the use of ozone micro/nanobubbles for disinfection of various kinds of microorganisms including Fusarium oxysporum, Pectobacterium carotovorum subsp. carotovorum [13], Coliform group [14], and Bacillus subtilis spores [15] demonstrated that ozone micro/nanobubbles were efficient for use as a disinfectant against those microorganisms.

As an application example in Thailand, it is being applied to shrimp farming in Chumphon province for exporting sushi-shrimps to Japan. In conventional procedures for preparing sushi-shrimps, chlorine-based washing is being exclusively used in the cleaning and sterilization steps, since chlorine solution is one of the most widely used disinfectants. However, chlorine is highly corrosive and remains to some extent. Moreover, the association of chlorine is possible to form carcinogenic chlorinated compounds (chloramines and trihalomethanes) in water [16]. The current concern about the side-effects of chlorine leads to establishment of the new techniques for maintaining quality while inhibiting undesired microorganisms. Therefore, ozone fine bubbles (O₃FB) technology was introduced for sterilization to all of treatment lines in the factory.

In order to make more convincing these outstanding sterilization effects associated with O₃FB water, it is necessary to get the substantial understanding on the sterilization effects of FB water of ozone gas. The purpose of this study is to introduce O₃FB technology as a sterilization method instead of using chlorine treatment to enhance the safety of food in the food industry.

II. METHODOLOGY

A. Preparation of ozone fine bubbles (O₃FB) water

Taking into consideration of the actual application of FB water, we conducted O₃FB water generation using reverse osmosis water (RO water) from original tap water at Rajamangala University of Technology Lanna (RMUTL). The RO water was treated by a reverse osmosis membrane filter of 0.1 nm, with de-ionization filter containing ion exchange resins and UV-light at room temperature (28°C).

O₂FB water was then generated by a corona discharge ozone generator with oxygen gas flow rate of 0.2 L/min which was connected to a RMUTL micro/nanobubble generator (RMUTL-KVM-10) at water flow rate of 10 L/min and operation pump pressure of 0.3 MPa. The O₂FB water generated from cavitation nozzle was re-circulated in the O₂FB water tank (Fig. 1). The concentration of dissolved ozone in water was measured by ozone test kit (Ozone Pack Test, Kyoritsu Rikagaku, Japan). The dissolved oxygen, oxidation-reduction potential (ORP), pH and temperature were also measured by a multi-meter water quality checker (Horiba U-54G, Japan) at 15, 30, 60 and 120 min of O₂FB generation time. The platinum electrode was used for ORP measurement. The
distribution and number density of the fine bubbles were measured by HORIBA LA-960A, showing approximately $10^8$ bubbles/mL having a peak around 200 nm.

B. Preparation of Escherichia coli suspension

*Escherichia coli* was obtained from the Department of Microbiology, Chiang Mai University, Thailand. Cell suspension of *E. coli* was prepared from stock culture which was inoculated into nutrient broth (NB) and incubated at 37°C, 100 rpm for 5 hrs. Cell suspension turbidity was measured by UV-VIS spectrophotometer (UV 1800 SPC, Macy, China) at OD 600 nm. The serial dilution and spread plate technique on nutrient agar (NA) were used to evaluate cell suspension at OD 600 nm (approximately $10^6$ CFU/ml).

C. Effect of fresh O$_3$FB water on inhibition of *E. coli*

One ml of cell suspension of *E. coli* was then inoculated into O$_3$FB water (9 ml) for 0, 15, 30, 60 and 120 min and incubated at room temperature for 10 min. Then, the cell suspension of *E. coli* from each O$_3$FB water was evaluated by spread plate technique on NA, incubated at 37°C for 24 hrs. The colony of *E. coli* was presented as the mean number of colony forming unit (CFU/ml). The experiments were carried out in triplicate. The values in the figures are averaged ones.

D. Effect of three days-storage O$_3$FB water on inhibition of *E. coli*

In consideration of the stability of O$_3$FB water, the sterilization effect of three days-storage O$_3$FB was investigated. O$_3$FB water (generation time 0, 15, 30, 60 and 120 min) were determined after 3 days storage in a dark bottle at room temperature (28°C). Similar to fresh O$_3$FB water, 1 ml of cell suspension of *E. coli* (approximately $10^6$ CFU/ml) was inoculated into three days-storage O$_3$FB water (9 ml) for 0, 15, 30, 60 and 120 min and incubated at room temperature for 10 min. Then, the cell suspension of *E. coli* from each three days-storage O$_3$FB waters was evaluated by spread plate technique on NA and incubated at 37°C for 24 hrs. The colony of *E. coli* was presented as the mean number of colony forming unit (CFU/ml). The experiments were carried out in triplicate. The values presented in the figures are averaged ones.

III. RESULTS

A. Dissolved ozone and oxygen in O$_3$FB water

The dissolved ozone concentration of all treatments was found below the detection limit (0.1 mg O$_3$/L) by using ozone test kit (Ozone Pack Test, Japan) (data not shown). Whereas, the dissolved oxygen (DO) concentration was found to gradually increase, 7.8, 12.7, 14.2, 14.6, and 15.7 ppm at 0, 15, 30, 60 and 120 min, respectively (Fig. 2).

B. Sterilization effect of fresh O$_3$FB water

Oxidation reduction potential (ORP) was measured for oxidation reaction of all treatments at 28°C. The highest value of ORP (845 mV) occurred in O$_3$FB water generated for 60 min. In contrast, the lowest number of *E. coli* (3.3 log$_{10}$CFU/ml) was found in the O$_3$FB water generated for 60 min (Fig. 3). These results may suggest that high value of ORP in O$_3$FB may provide a high microbial disinfection.

In addition, pH values of O$_3$FB water were determined during O$_3$FB generation. Generally, when CO$_2$ exists in the water, it can react with water to form carbonic acid. However, the concentration of CO$_2$ decreased during O$_3$FB generation because a lot of oxygen was introduced into the water. As a result, the pH of O$_3$FB water gradually increased with the O$_3$FB generation times.

It is interesting to see that lower pH (6.0-6.5) tends to raise the ORP, whereas, raising the pH to 7.0 lowers the ORP value (Fig.3).

The temperature of O$_3$FB water was measured at 0, 15, 30, 60 and 120 min of O$_3$FB generation time. The results showed that the temperature of O$_3$FB water increased with O$_3$FB generation time (Fig. 3), because of accumulated mechanical heat caused by a pump into re-circulating fluid.

C. Sterilization effect of three days-storage O$_3$FB water

After storage of O$_3$FB water in dark bottles at room temperature (28°C) for 3 days, the sterilization effect of the water was investigated. The effect of three day-storage O$_3$FB water on inhibition of *E. coli* is shown in Fig. 4. After 24 hrs incubation, the reduction of the number of *E. coli* cells was not observed. However, decrease of the number of *E. coli* cells was found in the fresh O$_3$FB water. These indicate that O$_3$FB generated in this study did not remain stable in water. The temperature might have affected the stability of fine bubbles.
IV. DISCUSSIONS

In our study, it is most likely that the efficiency of ozone generator from pure oxygen gas into ozone was pretty low, resulting in undetected dissolved ozone in all treatments of O3FB water. Moreover, the dissolved ozone was rapidly decomposed to O2 and •O at 28°C, resulting in increasing of DO (Fig. 2). Chuajedton et al. (2017) demonstrated that dissolved ozone concentration at 13°C was higher than that of 28°C [17], and in addition, higher temperature tends to decrease ozone solubility [18].

Oxidation-reduction potential is the potential (voltage) at which oxidation occurs at the anode and reduction occurs at the cathode. ORP has increasingly become a primary approach to standardizing water disinfection parameters, since ORP reflects the antimicrobial potential of the water [19]. The oxidation efficiency of O3FB at several generation times was affected by extension of hydroxyl radical (•OH) in water [17]. As the results, at the highest value of ORP (845 mV), the lowest number of E. coli (3.3 log10CFU/ml) was observed (Fig. 3). In microbial perspectives, since oxidation pulls electrons away from the cell membrane of bacteria, it will cause bacteria membrane to become destabilized and leaky. And eventually, destroying the integrity of the cell membrane leads to rapid death [19].

The pH values during O3FB generation showed the relation to ORP values. The results of this study conformed to other reports that the low pH values (less than 6.0) cause the increasing of ORP values. Whereas, the high pH value (more than 8.0) cause the decreasing of ORP value [19]. Moreover, the lower pH values lead to slow ozone decomposition in water [17].

As the temperature of water is strongly related to the ability of dissolved ozone in water, the lower temperature could increase the ability of dissolved ozone in water [17], also as is shown that the concentration of ozone microbubbles water showed the highest at 15°C compared to at 20°C and 30°C after 5 min. generation [13]. However, in our study, the temperature of O3FB water increased with O3FB generation time (Fig. 3). Therefore, the temperature control will be considered for the future research.

The stability of FB (micro/nanobubbles) is still not clarified. Therefore, the 3 days-storage O3FB water at 28°C was investigated. However, the sterilization effect was not observed. The previous study on the effect of temperature on stability of FB demonstrated that FB stored at 4°C are more
stable than those stored at room temperature due to the less rigid shell of microbubbles at high temperature [20].

V. CONCLUSIONS

O}_{3FB} water is shown to have sterilization effect on E. coli. The generation time of O}_{3FB} for 60 min at 28 °C by fresh O}_{3FB} used, provides the best results on the reduction of E. coli, which is related to the highest value of ORP. However, the sterilization effects of O}_{3FB} after three days-storage was not observed in this experiment. Therefore, in order to use O}_{3FB} water for sterilization, fresh O}_{3FB} water is more effective compared to the storage O}_{3FB} water. This finding will be useful for the practical use of O}_{3FB} as a sterilization technique to enhance the safety of food.

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