The Storage Effect of Ozone Fine Bubble Water on Sterilization of *Escherichia coli*

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Abstract - Sterilization effect of ozone fine (micro/nano) bubble water was studied, taking into account the "storage time". The number density of the fine bubbles showed approximately 10^9 bubbles/mL. The dissolved ozone concentration of O_3 fine bubble water was gradually decreased from 4.20 (fresh O_3 fine bubble water) to 3.35 (7 days storage of O_3 fine bubble water) mg DO_3/L. The effect of O_3 fine bubble water on sterilization of *Escherichia coli* was observed in the fresh O_3 fine bubble water condition. It was showed that the number of *E. coli* gradually decreased when the contact time increase. In addition, the number of *E. coli* was below the detection limit at the contact time 20, 25, and 30 min. In contrast, one to seven days storage of O_3 fine bubble water showed no sterilization effect of *E. coli*. The results of this study suggested that an application of fresh O_3 fine bubble water might be effective for sterilization. This finding will be useful for sterilization process in food industry.

Keywords – fine bubble, micro/nano bubble, sterilization, ozone, *Escherichia coli*

I. INTRODUCTION

At present, food safety issue has drawn great attention in the food industries of Thailand due to the concerning of consumers on their health benefits. Meanwhile food safety problems in terms of pathogenic microorganisms contamination have been repeatedly report [1]. Consumers have also become more critical of the use of synthetic additives to preserve food or enhance the safety of food [2]. As a traditional washing treatment in food processing, chlorine (Cl) solutions have been widely used as sanitizing agent [3, 4]. However, the association of chlorine in water is possible to form carcinogenic chlorinated compounds (chloramines and trihalomethanes) [3, 5]. Therefore, an alternatives method for sterilization of food is needed to improve the efficacy of washing treatments.

Fine bubble technology is an emerging and innovative technology applicable for food safety. Recently, fine (micro/nano) bubble water is preferentially being used for sterilization of microorganisms, in fishes, fruits and so on. In Chumpon shrimp factory, for example, ozone fine bubble water is being used instead of chlorine water in washing step. An application of ozone fine bubble water is not only control microorganisms but also useful for shorten the process time, improvement of taste and no chlorine remaining. As of Tak banana factory, they succeeded in sterilizing the crown parts of bananas using air and nitrogen fine bubble water. Generally, the banana exported to Japan was serious damage at the crown part by fungi during transportation. However, the used of the fine bubble technology in washing step was successful to prevent the banana from fungi during transportation to Japan. Also, coconut juice was found to be preserved freshness without any chemicals added by ozone fine bubble water treatment.

Among various fine bubble water, the O_3 fine bubble water has strong oxidative activity and can be applied to various water treatment processes including sterilization [6]. Previous researches revealed that O_3 fine bubble were efficient for sterilization of various kinds of microorganisms such as Coliform bacteria [7], *Bacillus subtilis* spores [8], *Fusarium oxysporum*, and *Pectobacterium carotovorum* subsp. *carotovorum* [9]. It was found that O_3 fine water has ample hydroxyl radicals measured by Electron spin-resonance spectroscopy (ESR) [10], In addition, some studies reported that microbubbles could accelerate the formation of the hydroxyl radicals during ozonation processes [11, 12, 13].

In previous study, the sterilization effect of ozone fine bubble water was investigated focusing on O_3 fine bubble generation time. The sterilization effect was conducted during O_3 fine bubble generation and at only day 3 storage [14]. However, the effect of sterilization by O_3 fine bubble water in terms of "storage time", the period since fine bubble is produced, is still unclear.

Taking into consideration of actual application of fine bubble water, the storage time of fine bubble water is found necessary to start killing bacteria. In order to make more convincing these outstanding sterilization effects associated with O_3 fine bubble water, it is necessary to get the substantial understanding on the effect of storage time of O_3 fine bubble water on sterilization. Moreover, the period since O_3 fine bubble water contact with microorganisms (contact time, CT) is found to be necessary for sterilization.

This study aimed to determine the effect of sterilization on *Escherichia coli* by O_3 fine bubble water focusing on the storage time and contact time.

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Presented at the 4th ISHPMNB 2019, in May 2019
Submitted: Aug. 1, 2019, Accepted: Dec. 19, 2019

II. Methodology

A. *Ozone fine bubble water*

Ozone fine bubble water was prepared according to the method of Saijai *et al.* (2019) with minor modification. The modified preparation of O_3 fine bubble was as follows. Reverse osmosis (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) before generating fine bubble water.

Ozone gas was then generated by a corona discharge ozone generator with oxygen gas flow rate of 0.15 L/min which was connected to a RMUTL micro/nano bubble generator (RMUTL-KVM-11) at water flow rate of 1.6 L/min and operation pump pressure of 0.8 MPa. The O_3 fine bubble water generated from cavitation nozzle was re-circulated in the water tank of O_3 fine bubble. During fine bubbles generation, the temperature was control approximately 10±1 °C. Distribution and number density of the fine bubbles were measured by HORIBA LA-960A (Japan). The concentration of dissolved ozone in water was measured by ozone test kit (Ozone Pack Test, Kyoritsu Rikagaku, Japan).

B. *Cell suspension of Escherichia coli*

Cell suspension of *Escherichia coli* was prepared from the stock culture by inoculation into nutrient broth (NB) and shaking (100 rpm) at 37 °C for 4 hr. The growth of *E. coli* was measured by UV-VIS spectrophotometer (UV 1800 SPC, Macy, China) at OD 600 nm. The ten-fold serial dilution and spread plate technique on nutrient agar (NA) were used to evaluate the number of cell suspension of *E. coli* at OD 600 nm (approximately 10^5 CFU/ml).

C. *Effect of ozone fine bubble water on sterilization of E. coli*

The effect of O_3 fine bubble water on sterilization of *E. coli* was investigated by using fresh O_3 fine bubble water and storage O_3 fine bubble water. One mL of cell suspension of *E. coli* was inoculated into the fresh O_3 fine bubble water (9 mL) with the contact time (CT), the period since O_3 fine bubble water contact with *E. coli*, at 0 (control), 5, 10, 15, 20, 25, and 30 min.

In consideration of the storage time of O_3 fine bubble water, the sterilization effect of one to seven days storage O_3 fine bubble water was investigated. The storage O_3 fine bubble water was kept in the dark bottles at 4 ˚C. Before testing, the storage O_3 fine bubble water was placed at room temperature (28 ˚C). Similar to the fresh O_3 fine bubble water experiment, 1 ml of cell suspension of *E. coli* was inoculated into storage O_3 fine bubble water (9 ml) with the contact time at 0 (control), 5, 10, 15, 20, 25, and 30 min.

The number of *E. coli* from fresh and 1-7 days storage O_3MNB water was evaluated by spread plate technique on nutrient agar (NA). The *E. coli* agar plates were then 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.

III. Results

A. *Number density, size, dissolved ozone and pH of ozone fine bubble water*

The number density of the O_3 fine bubbles generated by RMUTL micro/nano bubble generator (RMUTL-KVM-11) was measured by bubble analyzer (HORIBA LA-960A, Japan). The bubbles size was detected around 50-200 nm. with the peak at 100 nm. The number density of O_3 fine bubble was approximately 10^9 bubbles/mL.

Dissolved ozone (DO_3) concentration was measured using ozone test kit (Ozone Pack Test, Japan) for 7 days. The initial dissolved ozone concentration of O_3 fine bubble water was approximately 4.20 mg DO_3/L. The dissolved ozone concentration was gradually decreased from 4.20 (fresh O_3 fine bubble water; 0 day storage) to 3.35 mg DO_3/L (7 days storage O_3 fine bubble water). Whereas, the pH values of O_3 fine bubble water was stable at pH 5 during storage (Fig 1).

B. *Effect of ozone fine bubble on sterilization of E. coli*

In the fresh O_3 fine bubble water (0 day storage), the decreasing of the number of *E. coli* was observed when the contact time (CT) increasing. As a control (CT 0 min), 4.9

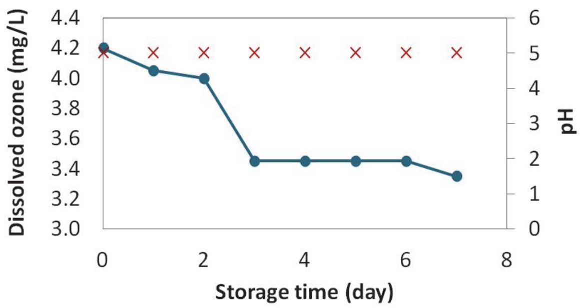

Fig. 1. Concentration of dissolved ozone (●) and pH (x) in ozone fine bubble water.

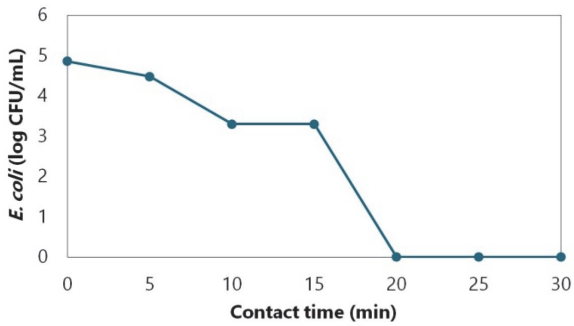

Fig. 2. The logarithm of the number of *E. coli* in the fresh ozone fine bubble water.

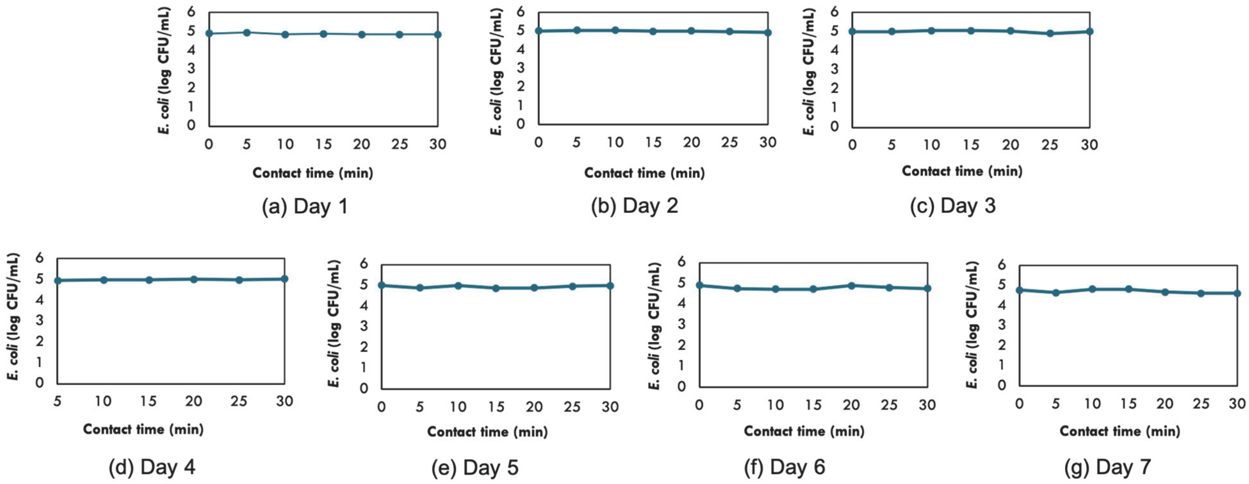

Fig. 3. The logarithm of the number of *E. coli* in 1 to 7 days storage of ozone fine bubble water (a-g), respectively.

log CFU/mL of *E. coli* was detected. The number of *E. coli* gradually decreased to 4.5 log CFU/mL and 3.3 log CFU/mL at CT 5 min, CT 10 and 15 min, respectively. The dramatically decreased of the number of *E. coli* was observed at the CT 20 min. At the CT 20, 25, and 30 min, the number of *E. coli* was below the detection limited (Fig. 2). These results showed the effectiveness of the fresh O_3 fine bubble water on sterilization of *E. coli*.

On the other hand, no sterilization effect of *E. coli* was observed in 1-7 days storage of O_3 fine bubble water. As a result, approximately 4.9 log CFU/mL of the number of *E. coli* was detected (Fig. 3). It could be concluded that no storage effect of O_3 fine bubble water on sterilization of *E. coli*. These results suggested that the application of O_3 fine bubble water for the purpose of sterilization will be the most effective when using the fresh O_3 fine bubble water.

IV. Discussion

This study is focus on the actual application of fine bubble technology in terms of sterilization. In order to enhance the safety of food in the food industry, it is necessary to get the substantial understanding on sterilization effect of fine bubbles. Among various kind of fine bubble water, the ozone fine bubble water has strong oxidative activity and can be applied to sterilization [3, 6].

The stability of dissolved ozone in water is related to the temperature of water. Previous study revealed that dissolved ozone concentration at 13 ˚C was higher than that of 28 ˚C [15], and in addition, higher temperature tends to decrease ozone solubility [9, 16]. In our study, O_3 fine bubble was generated under the temperature around 10±1 ˚C and was kept at 4 ˚C for a week. As a result, the small amount of ozone concentration was decreased (Fig. 1).

The effective sterilization effect of *E. coli* was occurred in the fresh O_3 fine bubble water (Fig. 2). The effective contact time for sterilization of *E. coli* was found after contact time for 5 min, and at the contact time for 20 min the number of *E. coli* was below the detection limited in the fresh O_3 fine bubble water. The results of this study conformed to other report that cell suspension of *E. coli* was inactivated after exposure time for 5 min, and the exposure time for 30 min obtained the best result in inactivate cell suspension of *E. coli* [15].

However, the sterilization of *E. coli* was not observed in the 1-7 days storage O_3 fine bubble water. Generally, fine bubbles are decrease in size and subsequent collapse under water [17]. Hydroxyl (•OH) radical, which is a strong oxidant are generated from collapse of fine bubbles. The shrinking rate of the collapsing fine bubbles was approximately complete over a time course of tens of seconds [10], whereas ultrafine bubbles, the bubbles showing the size smaller than that of the fine bubbles, are stable remaining in the water longer. In the storage O_3 fine bubble water experiment, the number density of fine bubbles might be decreased rapidly after O_3 fine bubbles generation. Therefore, no hydroxyl (•OH) radical was generated from collapse of fine bubbles in the storage O_3 fine bubbles. As a result, no sterilization of *E. coli* was observed.

V. Conclusion

Ozone fine bubble water without storage is shown to have sterilization effect on *E. coli*. The fresh O_3 fine bubble water at the contact time of 20-30 min provides the best results on the reduction of *E. coli*. However, the sterilization effects of 1-7 day storage of O_3 fine bubble water was not observed in this experiment. Therefore, in order to use O_3 fine bubble water for sterilization, the fresh O_3 fine bubble will be the most effective application. These finding will be useful for introduction of O_3 fine bubble technology to practical use as an alternatives sterilization method instead of using chemical agent treatment to enhance the safety of food.

ACKNOWLEDGMENT

This work was supported by the center of excellence on high voltage plasma and micro/nanobubble for agriculture and aquaculture, Rajamangala University of Technology Lanna (RMUTL), Chiang Mai, Thailand. The authors thank Department of Microbiology, Chiang Mai University, Thailand for supplying the *E. coli* used in this study.

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