Effects of Oxygen Micro Bubble Water on the Recovery Process of Tilapia Fry Transportation at High Stocking Density and Long Distance

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Abstract—Effects of oxygen microbubble (O_2-MB) water on the recovery process of Nile tilapia fry after 10 hours transportation at high stocking density (1,000 fish/bag) were studied. Three different kinds of water using the same hatchery water, namely, normal farm water (Water #1), O_2-MB water generated for 1 hour (Water #2), and O_2-MB water generated for 1 hour and left 24 hours (Water #3) were used in transporting bags. Water quality, bacterial population and survival rates were also investigated throughout experiments. After 10 hours transport, fishes were released into recovery tanks that were oxygenated by three different aerators, namely, an air blower, an air-MB and an O_2-MB in a circulation system to investigate survival rates for 3 days. The results revealed that the tilapia fry loaded in Water #3 had a higher survival rate than Water #1 and #2. It also showed the highest survival rate in recovering tank aerated by an O_2-MB, and the lowest by an air blower. O_2-MB water in both the transportation and the recovery process is found to help, eventually, improve fish survival rates.

Keywords—Microbubble, high stocking density, recovery process, tilapia, fish transportation

I. Introduction

Higher stocking density, longer time, and higher survival rates are strongly preferred for tilapia fry transportation by hatcheries in Thailand. Nevertheless, fish mortality is still the critical problem in the traditional transportation system of fish fry, mainly due to the accumulation of toxic waste, such as ammonia, decrease in pH due to increase of carbon dioxide, depletion of oxygen in the water during transportation [1].

Among them, oxygen dissolution in the water is the limiting and important factor for fish health in transportation. In order to improve fish survivability, an emerging technology, i.e., Oxygen microbubbles (MB) water was applied in the fish transportation.

Fine bubbles (FB) technology consisting of micro and nano size bubbles, i.e., micro bubbles (1μm<MB<100μm dia.), and ultra-fine bubbles (UFB<1μm dia.) is now rapidly emerging as an innovative technology.

It is now being used in various applications in, such as agriculture, aquaculture, sterilization and wastewater treatment [2] due to their distinctive characteristics, such as large specific surface area, negatively charged surface, and the generation of highly reactive free radicals [3-6]. One of remarkable features of MB enables dissolved oxygen levels as high as possible to keep fishes and all live happy and healthy [7].

The previous research [8] studied Oxygen microbubbles (O_2-MB) application to 8 hours Nile tilapia fry transportation at 500, 600, 700, 800 fish/bag together with the conventional method in Thailand (mostly, about 500) fish/bag less than 6 hours transportation).

Although, the results show 100% of survival rates for even the highest stocking density of 800 for both traditional and O_2-MB cases, during 3 days recovery processes with a conventional aeration system, approximately 1% mortality was seen for both cases. However, it is found that O_2-MB water implied larger improvement of the survival rate than the conventional even after recovery process.

In this study, we have challenged more higher stocking density from usual 500 fry/bag to 1,000 fry/bag in 4L O_2-MB water filled with 6L pure oxygen in a 10L sealed plastic bag, longer transportation time to 10 hours, and use of O_2-MB water in the recovery processes, to examine potential application of O_2-MB water to recovery process, particularly, at higher stocking density and longer distance. In the experiments, the water quality change, bacterial population and survival rate were investigated.

II. Methodology

A. *Preparation of O_2 microbubble water before fish loading*

The KVM20 microbubble generator was used in this study which was developed by Rajamangala University of Technology Lanna (RMUTL), Thailand by using a cavitation method. The KVM20 can produce MB with diameters of 4-13 μm as shown in Fig.1, and MB disappears in a few minutes.

The present experiments consisted of 3 parts for evaluating the effects of O_2-MB water, namely, before fish loading, after transportation, and recovery process.

The water for transportation was supplied from the hatcheries (Phitsanulok province, Thailand), and tap water was used for recovery process, after leaving for one week to get rid of chlorine.

The O_2-MB water in a 200L plastic tank was generated by KVM20 at a water flow rate of 20L/min with an oxygen gas flow rate of 2 L/min at pressure of 0.4 MPa, for 1 hour, and evolution of water quality was measured for 24 hours afterwards, such as dissolved oxygen (DO), temperature and pH, as well as total bacteria counts.

This water was made just for taking into consideration of easier practical applications by hatcheries, and used as Water#3 in the fish transportation experiment. Water#2 was

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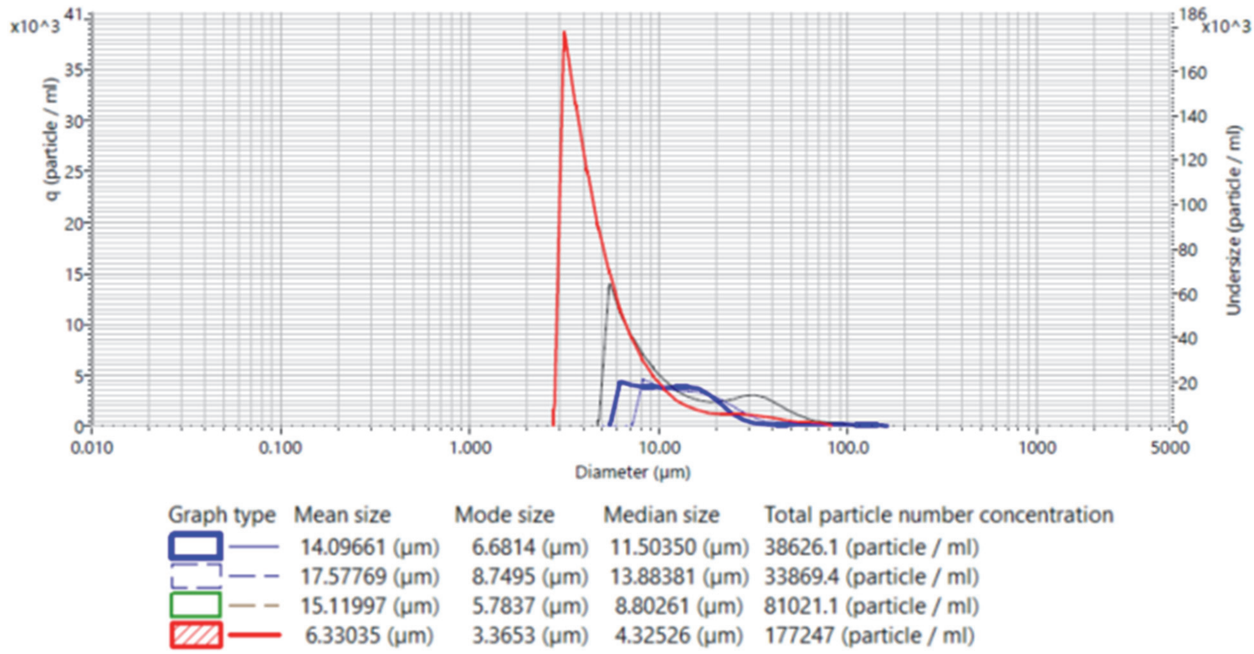

Fig.1. The performance of KVM20 microbubble generator developed by RMUTL at a water flow rate of 20L/min with O_2 gas flow rate of 1.2L/min, at pressure of 0.4 MPa by generating for 20 min in a recirculation system. Distributions measured every 5 minutes by the particle-size analyzer, HORIBA LA-960A.

produced by the same procedure at the laboratory and brought to hatchery located about 30 minutes-drive away, and the initial DO was measured at the hatchery farm just before fish loading to be 25mg/L.

B. *Experimental design for fish transport*

Nile tilapia fry (total length 1.89 ± 0.10 cm and weight 0.88 ± 0.02 g (mean ± SE)) were used in this study and they were starved a day before transportation in order to decrease discharge. One thousand tilapia fish fry were loaded in a 10L transport plastic bag which contains 4L water together with 6L pure oxygen gas at atmospheric pressure. The experiments were made in triplicates.

Three kinds of water made from the same hatchery water were shown below for 10 hours Nile tilapia fry transportation experiments:

Water #1: normal hatchery farm water (conventional)
Water #2: O_2-MB water generated for 1 hr
Water #3: O_2-MB water generated for 1 hr and left for 24 hrs

Water quality parameters, DO, temperature, pH (by Horiba U-54G multi-parameter water quality checker), and total ammonia (by Aquacare 2000.4 Para ammonium test kit, Para test) were measured before and after transportation as well as total bacterial counts.

Total bacterial counts were determined by the spread plate technique. The 10-fold dilutions of water samples were prepared and 0.1 ml of each dilution was transferred into duplicate plates of Nutrient Agar (Difco). After incubating at 30 °C for 18-24 hours, the plates having 30-300 colonies were counted for viable counts.

C. *Experimental design for fish recovery process after transportation*

After 10 hours transportation, fishes in each bag were released separately into nine 200L recovery tanks filled with almost chlorine-free tap water.

Recovery processes were conducted using continuous oxygenation by three different aerations, namely, an air blower (conventional), an air-MB and O_2-MB generator, alternately produced, in a circulation system. Air-MB generated for 1 hour at 6 am, 12 am, 6 pm and 12 pm, and O_2-MB generation for 1 hour was made at 9 am and 4 pm. Survival rates were estimated by counting the number of live fishes during 3 days recovery processes.

III. Results

A. *Water quality change of O_2 microbubble water before fish loading*

Water quality change of O_2-MB is shown in Fig.2 for 24 hours before fish loading. The initial DO, temperature and pH in the original hatchery water were, respectively, 5.5mg/L, 26.8°C and 7.4, and after 1 hour running of an O_2-MB generator with 200L water, they increased to 37mg/L, 40.7°C and 8.0, respectively. Then, after stopping the generator, all parameters were found to reduce drastically, and became stable in 6 hours, to 6 mg/L, 25.2 °C and 6.7, respectively (Fig 2).

Regarding initial pH increase in Fig.2-c, it is attributed to the small amount of dissolved minerals in the hatchery underground water.

Total bacteria counts were found to increase after generating O_2-MB for 15 min, and then sharply reduced in 60 min later. After stopping O_2-MB generation, the bacteria was found to increase again gradually (Fig.2-d), due probably to that the aerobic bacteria could grow more favorably in relatively higher DO water.

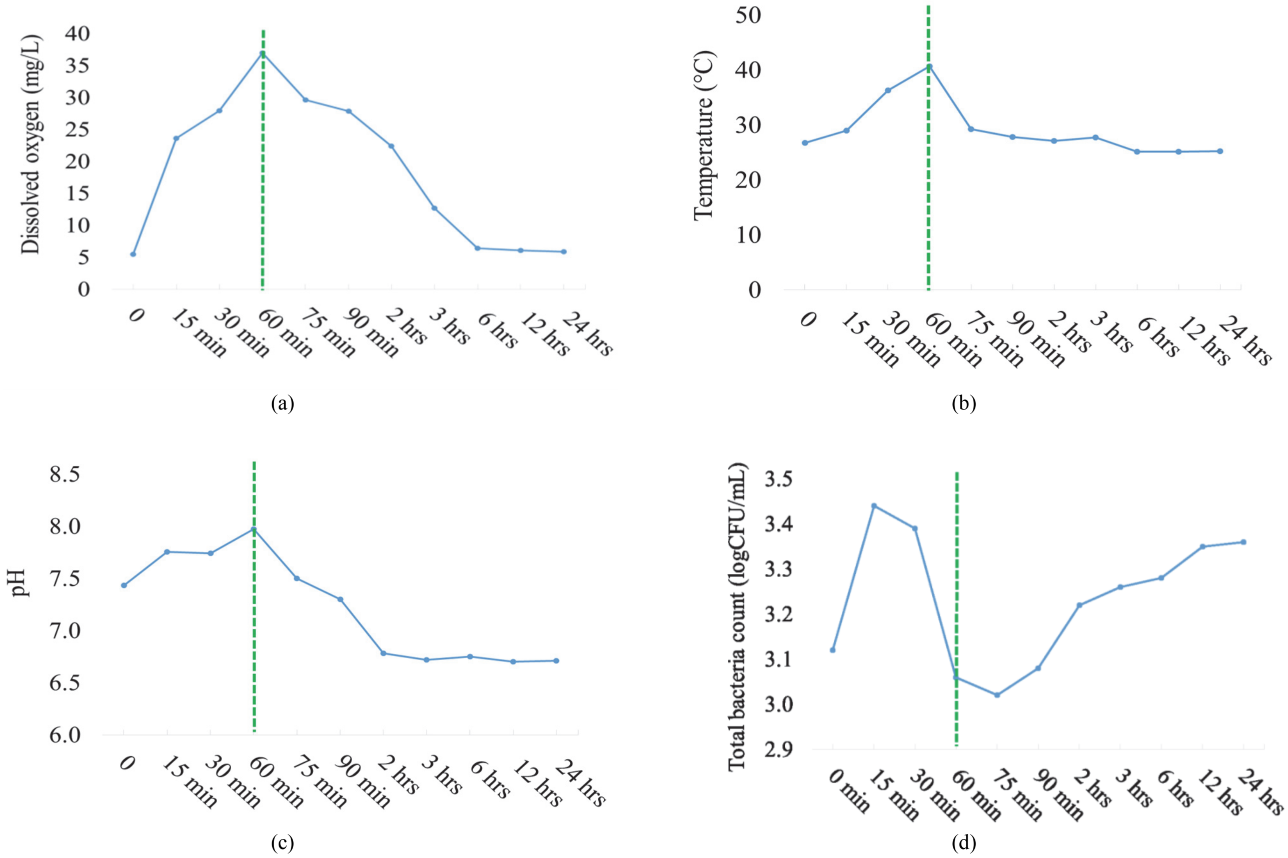

Fig. 2. Water quality (a-c), and total bacteria count (d) in O_2-MB water generated for 1 hour and then left 24 hours before fish loading. (horizontal not to scale)

B. Water quality changes of fish transportation by using O_2 microbubble water

Water quality before and after transportation were measured and shown in Fig.3. It is found that pH decreased in all treatments after transport. Dissolved oxygen level for Water#2 and #3 remained still high after 10 hours transportation compared with Water#1, which eventually lead to aerobic bacteria increase and ammonia increase due to enhanced fish activity.

As of DO in Water #1, #2 and #3, they changed from initial 7.8 to 4.1, 25.7 to 10.3, and 17.8 to 10.6 mg/L, respectively (Fig.3-a). Total ammonia level in Water #1 showed lower than Water#2 and #3 due to depressed fish activity as well as bacteria number (Fig.3-c,d).

It is shown consequently that higher DO can increase not only fish activity, but also number of aerobic bacteria in Water #2 and #3. The latter could be useful in decomposing toxic ammonia into nitrite and nitrate eventually.

C. Survival rate of 10 hours fish transport and 3 days recovery process

After 10 hours transportation, the results revealed that Water#3 had a higher survival rate than Water #1 and #2 (Table I). Although initial DO was very high for Water#2, the survivability rate was the lowest, probably due to much stresses caused by rapid change of DO in water.

Then, fishes were released into recovery tanks which were aerated, respectively, by an air blower, air-MB and O_2-MB generator in circulation system for 3 days, and the DO level in each tank measured were 5～6, 7～8 and 11～12 mg/L, respectively. Results clearly showed that when aerated by O_2-MB, the survival rates for Water #1-#3 were highest in recovery tanks, then followed by air-MB, and air blower as shown in Table I(mean ± sd).

TABLE I
SURVIVAL RATES (%) OF TILAPIA FRY AFTER 3 DAYS RECOVERY PROCESS BY USING DIFFERENT AERATORS IN CIRCULATION SYSTEM (MEAN ± SD)

Water	After 10 hrs transport	Recovery process		
		Air blower	Air-MB gen.	O_2-MB gen.
#1	95.2 ± 8.6	85.5 ± 9.2	89.8 ± 7.1	94.9 ± 7.2
#2	92.4 ± 9.7	72.3 ± 11.2	82.9 ± 6.3	92.2 ± 3.5
#3	97.5 ± 7.5	88.5 ± 8.6	90.8 ± 5.6	95.5 ± 5.5

IV. DISCUSSIONS

A. Water quality change of O_2 microbubble generated before fish loading

Effects of O_2-MB on water quality change and survival rates of tilapia fry in transport and recovery processes were studied. So long as our experiments are concerned, it was found that DO, temperature and pH increased during O_2-MB

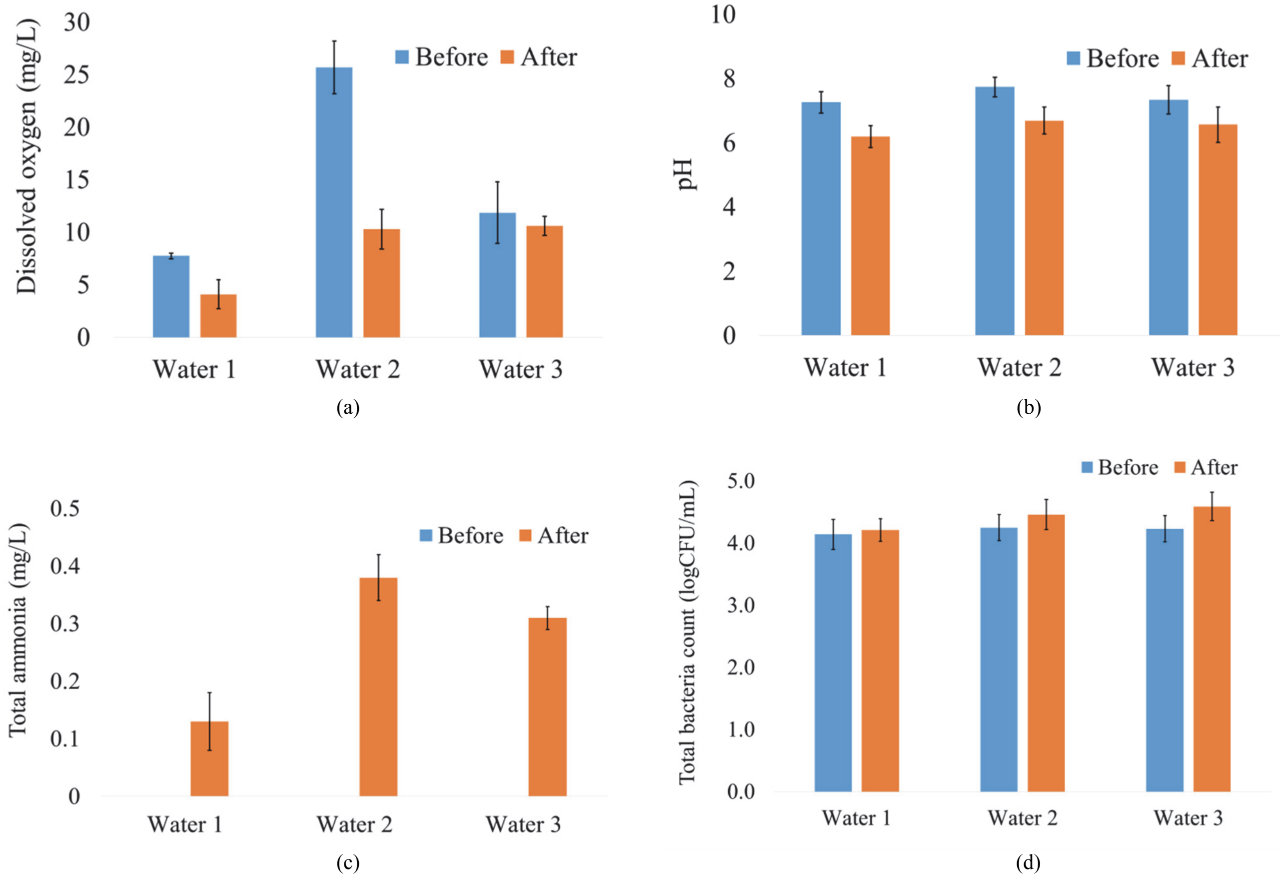

Fig.3. Water quality changes (a-c) and number of bacteria (d) during tilapia fry transport before loading fish and after 10 hours transportation.

generation for 1 hour. Due to excellent MB ability to enhance high gas concentration in solution, the O_2-MB increased DO very rapidly in water. The temperature rise is simply due to the generated heat related to pump power, and cooling will be needed in the future.

As of pH increase during O_2-FB production, it is well known due to the oxidation of small amount of dissolved minerals, such as, Fe, Mn, V and so on to form hydroxides either in the mineral water or in the ground water [9], i.e., hatchery water.

Furthermore, MB can either sterilize or stimulate aerobic microorganism activity. In this study, the result of total bacterial count presumed that some aerobic bacteria can grow under enhanced DO, and killed due to OH radicals generated during MB generation for 1 hour [10].

After 60 minutes O_2-MB generation, aerobic bacteria start growing again under relatively high DO and none OH radical conditions leading to rapid consumption of DO, and to increase CO_2 release, resulting in pH decrease.

B. *Water quality change of fish transportation by using O_2 microbubble water*

Fish fry transportation is one of the most difficult aspects for fish culture in causing stress responses [11] due to crowding from high density stocking and poor water quality due to nitrogenous waste, which have harmful effects on both growth and welfare [12].

After 10 hours fish transport, water qualities in all treatments were shown still in suitable ranges for fish survival. As predicted, DO level and pH decreased more compared with those at fish loading for all treatments (Fig.3-a,b).

DO level is due to oxygen consumption by fish respiration, and biochemical activity by microorganisms. During transportation, carbon dioxide (CO_2) was released from fishes, and form carbonic acid (H_2CO_3), eventually, to lower pH [13].

Moreover, ammonia levels in Water#2 and #3 were found also higher than in Water #1(Fig.3-c). It is found that O_2-MB in Water #2 and #3 can promote the number of aerobic bacteria (Fig.3-d) due probably to enhanced oxygen content without further OH radical generation. This could speed up chemical reaction in converting nitrogenous waste to ammonia in water.

C. *Survival rate in fish transport for 10 hours and recovery process for 3 days*

Survival rates of fish fry generally increase with decreasing stocking density, and transportation time. At high stocking density loaded in transporting bags, since fishes have to compete for space and available DO, water quality like temperature and DO affect strongly fish survival rates [14].

In the Nile tilapia fry transport at high stocking density of 1,000 fish/bag under different water for 10 hours transportation, the survival rates were found to be pretty high in the range of 93 to 98% (Table I). The highest survival rate (98%) was achieved for Water #3.

On the other hand, Water #2 showed the lowest survival rate which might have been, as aforementioned, due to stress response from large and quick changes of water quality, namely, DO and temperature [15] from 5.5 to 37 mg/L, 26.8 to 40.7 °C, respectively, as shown in Fig.2-a,b.

So, it is most likely that these parameters have affected the physiological changes of fishes from stresses. Besides, the massive amounts of floating micro bubbles occurred in Water #2, which could affect fishes because gasses escape from the supersaturated water by diffusing into the blood or other body fluids of the fishes.

Bubbles are predicted to form inside capillaries and under the skin known as gas bubble disease [16].

In the recovery process, our study indicated that O_2-MB aeration can improve survival rates due to increased dissolved oxygen in water.

For actual application of the present method, i.e., very high stocking density as high as 1,000 fish/bag, for 10 hours transportation, it would be most preferable for hatchery people to use, namely, 4L farm water with 6L pure oxygen gas for transportation, and then in the recovery process, tap water or farm water aerated by air MB by an MB generator.

It is to be noted that this MB generator can be used as an efficient aerator also for enhancement of dissolved oxygen level in the farm ponds.

Consequently, MB generators are found to be very useful in increasing not only final survival rates of tilapia fry in the transportation including recovery processes, but also can keep high the dissolved oxygen level of fish ponds during fish culture as well.

V. Conclusion

O_2-MB water applying to Nile tilapia fry transportation at high stocking density (1,000 fish/bag) for 10 hours is found to provide a slightly higher survival rates when compared with the conventional method. However, use of O_2-MB aerated in the water for 3 days recovery process is found to significantly improve a survival rates of tilapia fry after 10 hours transportation.

Acknowledgments

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