

Dissolved Oxygen, Temperature, and Total Ammonia Nitrogen Management of *Penaeus vannamei* Postlarvae 10 Hatchery Using Nanobubble Technology

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Abstract: Nanobubble technology is known to be able to manage the water quality of aquaculture. This study aimed to measure and compare the water quality of *Penaeus vannamei* postlarvae (PL) 10 using nanobubble technology and conventional treatments. The PL densities were 2,000 individuals, consisting of the control group (without nanobubble treatment) and nanobubble treatment groups with DO levels equal to 16 and 22 mg/l. Three replications and 24-hour measurements for each group were conducted. The measured water quality includes dissolved oxygen (DO), temperature, and total ammonia nitrogen (TAN). The results confirmed that nanobubble has promoted the water quality by increasing DO and reducing temperature and TAN. The water quality under nanobubble level at 22 mg/l was improved in comparison to control. The 24-hour average of DO, temperature, and TAN of nanobubble treatment group were 10.52 mg/l (95% CI: 9.86, 11.2), 24.6 °C (95% CI: 24.5, 24.7), and 2.35 mg/l (95% CI: 0.86, 3.84). Meanwhile, the 24-hour average of DO, temperature, and TAN for control were 9.94 mg/l (95% CI: 9.44, 10.4), 24.9 °C (95% CI: 24.8 to 25) and 2.58 mg/l (95% CI: 0.91, 4.25). To conclude, using 24-hour nanobubble can increase DO by 6%, and reduce the temperature and TAN by 2% and 9%.

Keywords: DO, nanobubble, PL10, TAN, temperature

Introduction

There are several water quality properties in aquaculture that should be taken into considerations. Those properties are dissolved oxygen (DO), pH, temperature, and total ammonia nitrogen (TAN). Currently, several measures have been developed to manage water quality, including the nanobubble technology. The principle of this approach is the changes and modifications of aquaculture systems to increase the concentration of DO in cultivation water, which is achieved by supplying DO to the water.

Nanobubble is an ultra-small gas bubble in liquid with a diameter of micron and submicron-order. It is characterized by its slow buoyancy, negative surface charges, free radical formation, and its ability to increase water molecule mobility (Ohmori et al., 2015). Nanobubbles in water are generated using decompression, gas-water circulation, ultrasonic waves, and small-porous-glass membrane (Ohmori et al., 2015; Temesgen, Bui, Han, Kim, & Park, 2017). Mahasri et al. (2018) have observed the increase of DO from 6.5 to 25.0 mg/L in cultivation water using nanobubble. The use of nanobubble for shrimp hatchery has been studied by Wang Yangcai, Liu Youyu (2018) as well. They found that nanobubbles can effectively improve the DO in water and maintain DO for a longer period. Under this nanobubble treatment, the average DO levels were recorded at 7.76% higher than the control. According to Wyban, Walsh, & Godin (1995), temperature is one of the key factors for shrimp growth. In addition, TAN level as a result of high rates of prawn excretion and feed loading

can reduce the performance of hatchery productivity (Valencia-Castañeda et al., 2018). Nonetheless, current research on the performance of nanobubbles in water quality have only been focusing on DO and temperature properties.

The novelty of this research is its inclusion of the total ammonia nitrogen measurement, instead of using only nanobubble treatments. Likewise, this study aimed to measure and compare the water quality properties of *P. vannamei* PL 10 hatchery using nanobubble technology and conventional treatments.

Materials and Methods

The study was conducted from 02 to 04 June 2020 in a hatchery in Serang, Indonesia. *P. vannamei* PL 10 were cultured and weighed at 0.04 g with the total length of 7.5-11 mm. The nanobubble machinery NB S-2, which was developed by Nanobubble Karya Indonesia Ltd., South Tangerang, Indonesia, and patented by Pusat Penelitian Fisika LIPI No. P00201903600, was used to generate oxygen bubbles with DO levels from 16 to 22 mg/L. The treatments used in these groups consist of control (without nanobubble) and treatment groups added with nanobubble equal to 16 mg/L (nano 16) and 22 mg/L (nano 22). The nanobubble preparations were conducted by operating a nanobubble generator (2 horsepower) with oxygen flow set to 0.4 L/min in a 300 L acclimated seawater storage container until the DO reached the designated levels of 16 mg/L and 22 mg/L. The nanobubble treatment was not applied for the control container. In addition, *P. vannamei* was placed in closed system using plastic bags 20x40 cm with a density of 2,000 PL10 individuals. Three replications were undertaken for the control and treatment groups.

The measured water quality properties include dissolved oxygen (DO), temperature, pH and total ammonia nitrogen (TAN). The DO and temperature were measured with YSI 550A, pH with YSI pH100, and TAN using Hanna Ammonia Test Kit for Sea Water. Three replications of water quality measurements were conducted at 8 and 24 hours.

The data were analyzed to calculate the mean \pm standard error and confidence interval (CI) at 95%. This analysis was applied to DO, temperature, pH, and TAN data. The analysis of these water quality indicators in control and treatment groups (nano 16, nano 22) were compared and assessed using ordination plots.

Results

All water quality properties were changing after 24 hours of observation, both in control and nanobubble treatments. First, a reduction was observed for DO and pH, while temperature and TAN were increasing. In 8 hours, the DO for control, nano 16, and nano 22 treatment groups were 13.01 ± 0.79 mg/L, 11.68 ± 1.62 mg/L, and 13.14 ± 1.23 mg/L, respectively. In the next 24 hours, the DO reduced to 9.95 ± 0.62 mg/L (control), 9.01 ± 0.94 mg/L (nano 16), and 10.52 ± 0.83 mg/L (nano 22) (Figure 1). Nonetheless, the group that was treated with nanobubble DO at 22 mg/L always had the highest DO compared to control.

An increasing water quality trend was observed for temperature and TAN. In 8 hours, the temperature for control, nano 16, and nano 22 treatment groups were $20.96 \pm 0.06^\circ\text{C}$, $20.97 \pm 0.45^\circ\text{C}$, and $20.3 \pm 0.26^\circ\text{C}$, respectively. After 24 hours, the temperature increased to $24.90 \pm 0.10^\circ\text{C}$ (control), $24.7 \pm 0.17^\circ\text{C}$ (nano 16), and $24.6 \pm 0.01^\circ\text{C}$ (nano 22) (Figure 2). Despite increasing, the nano 16 and nano 22 treatment groups had lower temperature compared to control. In addition, a decreasing water quality trend was also observed for pH level. In 8 hours, the pH for control, nano 16, and nano 24 treatment groups were 7.19 ± 0.14 , 6.99 ± 0.08 , and 7.01 ± 0.09 , respectively, while in 24 hours pH decreased to 6.65 ± 0.03 , 6.59 ± 0.01 , and 6.54 ± 0.05 (Figure 3).

The same trend was also observed for TAN. After 24 hours the TAN for control and treatment groups increased almost by four folds. Despite the increment, TAN measured from nano 16 and nano 22 treatment groups had

lower values compared to control (Figure 4). The ordination plots (Figure 5) support and explain clearly that the treatment groups always had different values of DO, temperature, pH, and TAN in comparison to the control.

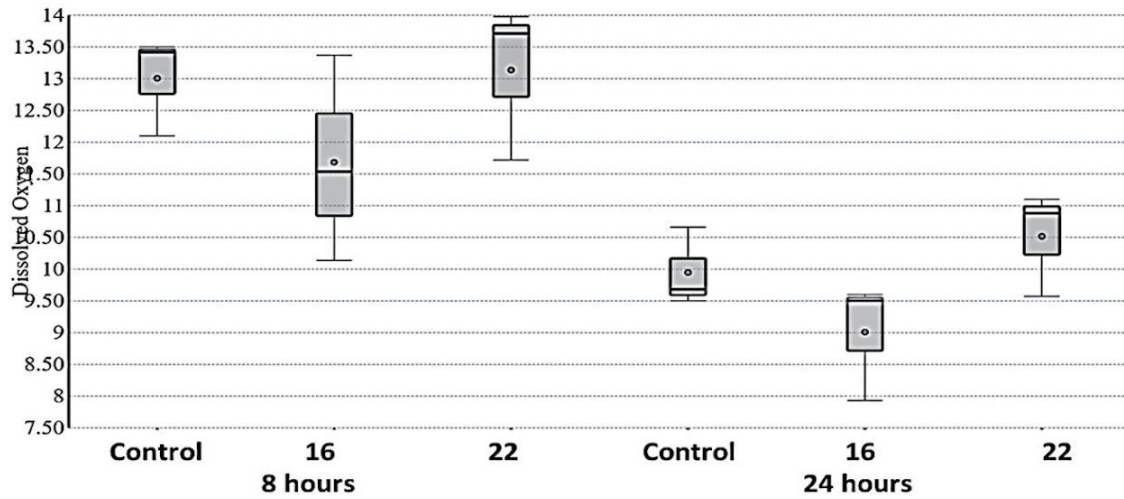


Figure 1. Dissolved oxygen (DO) mean levels for control, treatments (nanobubble levels: 16 mg/L and 22 mg/L) at 8 and 24 hours under PL10 with density of 2,000 individuals.

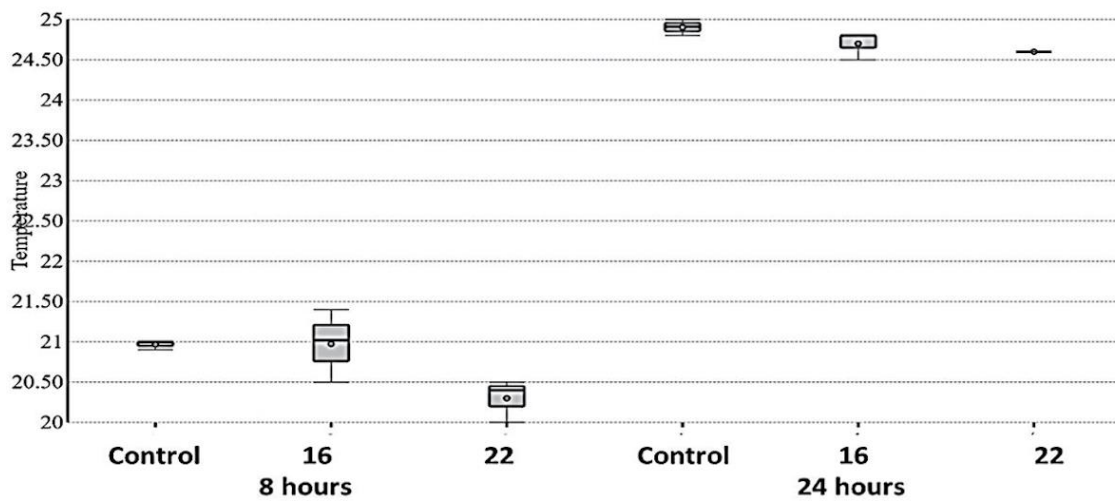


Figure 2. Temperature mean levels for control, treatments (nanobubble levels: 16 mg/L and 22 mg/L) at 8 and 24 hours under PL10 with density of 2,000 individuals.

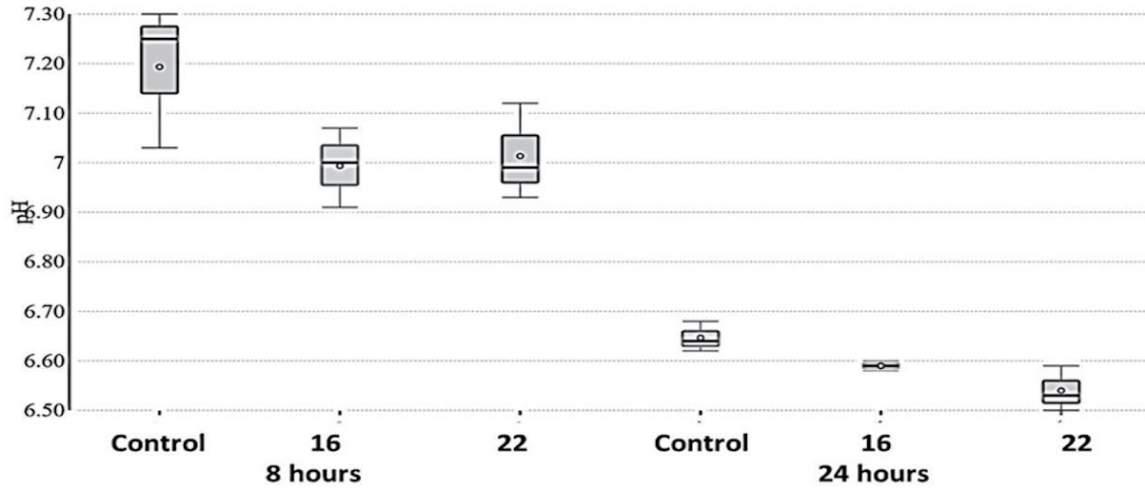


Figure 3. pH mean levels for control, treatments (nanobubble levels: 16 mg/L and 22 mg/L) at 8 and 24 hours under PL10 with density of 2,000 individuals.

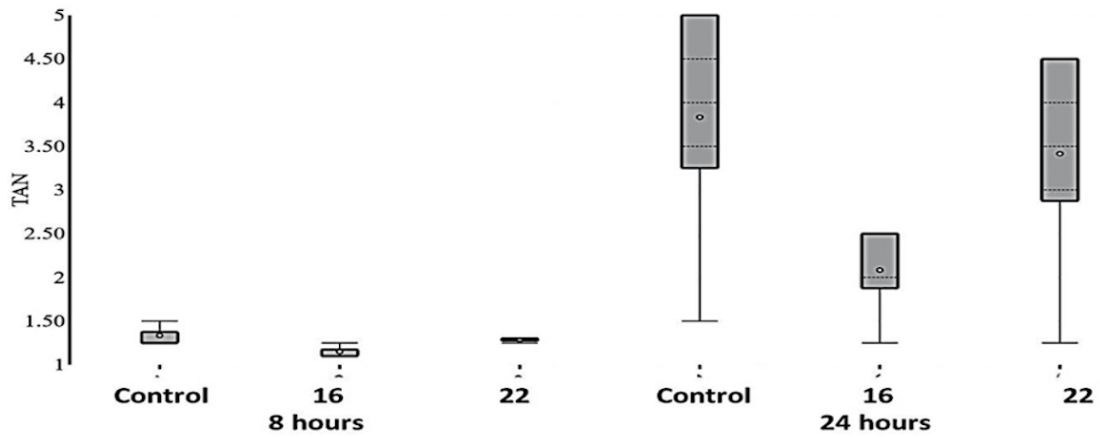


Figure 4. Total Ammonia Nitrogen (TAN) mean levels for control, treatments (nanobubble levels: 16 mg/L and 22 mg/L) at 8 and 24 hours under PL10 with density of 2,000 individuals.

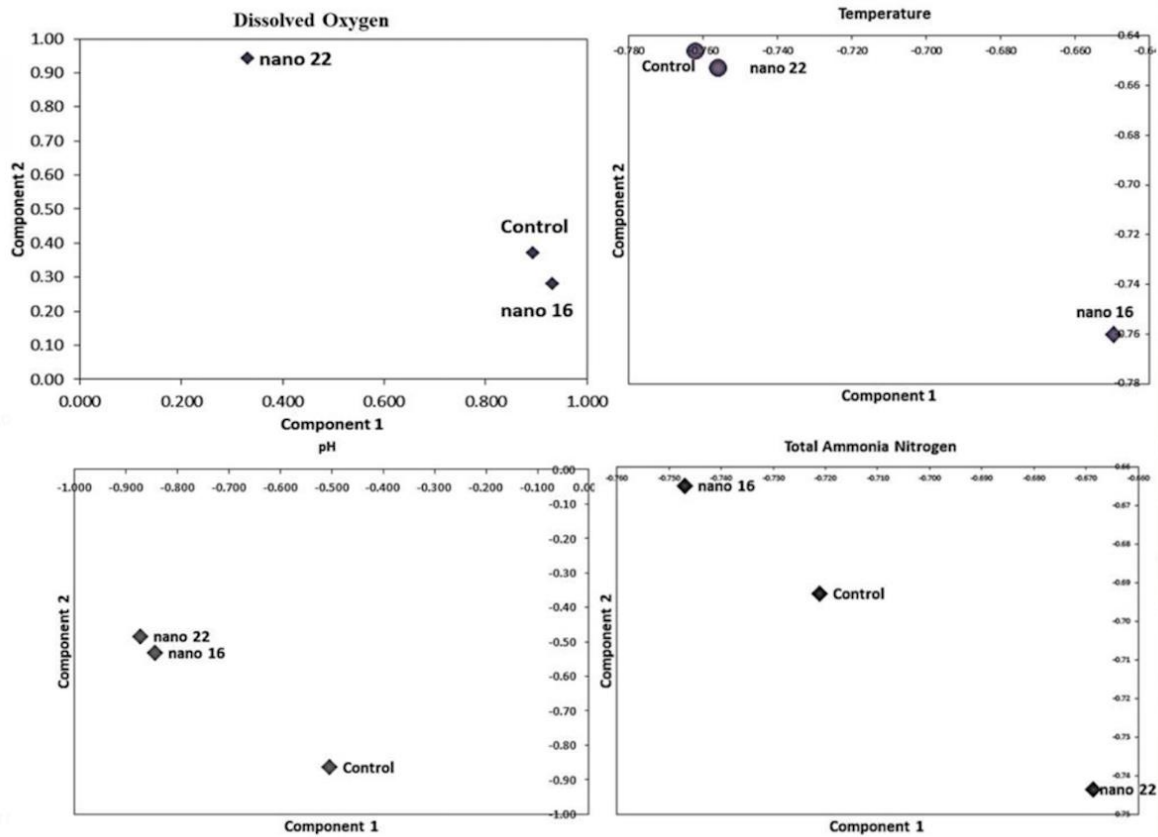


Figure 5. Ordination plots of dissolved oxygen (DO), temperature, pH, and total ammonia nitrogen (TAN) levels for control, treatments groups nano16 (DO: 16 mg/L) and nano 22 (DO: 22 mg/L) under PL10 with density of 2,000 individuals.

Discussions

The reductions of several water quality properties in this study, such as DO, were also reported in other experiments using nanobubble. Water that was treated with nanobubble did not change and was stable at an average level of 13.5 mg/L for about 48 hours. After a few days, the DO level reached the saturation equilibrium and reduced to 9.1 mg/L (Tekile, Kim, & Lee, 2016). Likewise, Galang et al. (2019) reported that the DO reduced from 10.8 ± 0.07 mg/L to 8.25 ± 0.09 mg/L after 48 hours. The DO reductions are related to DO consumption by reared white shrimp *P. vannamei* as several literatures have discussed (Vinatea, Gálvez, Venero, Leffler, & Browdy, 2009). The shrimp DO consumption was a function of salinity, temperature, stocking density, and wet weight as stated by Vinatea, Muedas, & Arantes (2011) that the increase in shrimp weight and stocking density have a multiplying effect on the DO consumptions. At a stocking density of 10 shrimps/m², wet weight of 5 g, temperature of 20°C, and salinity of 25 and 37 ppt, a low oxygen consumption was observed, and it was equal to 0.01 mg/L/hour. Nonetheless, when the density was multiplied to 20 shrimps/m², wet weight of 20 g, temperature increased to 30°C, and salinity of 1 ppt, a high DO consumption was observed, and it was equal to 1.13 mg/L/hour. In a hatchery in East Java, Supriatna, Marsoedi, Hariati, & Mahmudi (2017) reported that other external environmental factors have influenced the DO. They observed that DO was positively correlated with temperature and pH, while DO negatively correlated with salinity, phosphate, organic matter, carbonates, and bicarbonates.

Another concern regarding hatchery water quality properties was related to temperature and TAN matters. Temperature recorded in this study was still within the ranges of studies conducted by other researchers. The reported temperature in those studies were 20-32°C for Cheng, Wang, & Chen (2005), 28-31°C for Djunaedi,

Susilo, & Sunaryo (2016), and 27°C for Sahrijanna & Sahabuddin (2014). The observed dynamics and increasing trends of temperatures were related to the chemical properties of water molecules, solar radiation, air temperature, and the water temperature passing through the treatment units. Water consists of atoms and molecules and these mass of matter to which energy is added are vibrating faster, rapidly, and move slightly farther apart. As a result, the movements of atoms and molecules generate energy and heat content that raise the temperature over time.

Increasing TAN is another important water quality for PL10 hatchery. Djunaedi et al. (2016) observed the increasing trends for ammonia and nitrite were 0.04-0.12 mg/L and 0.1-0.3 mg/L, respectively. Sahrijanna & Sahabuddin (2014) reported the ammonia to be equal to 0.53 mg/L and nitrite at 2.32 mg/L. TAN is associated with PL10 hatchery and it resulted from high rates of prawn excretion and feed loading. High TAN levels can adversely affect hatchery productivity (Valencia-Castañeda et al., 2018). Furthermore, increasing TAN levels recorded in this study were comparable to study by Zhou & Boyd (2015). They found that TAN levels fluctuate greatly over time and had levels between 5 and 15 mg/L.

One of the advantages of utilizing nanobubble is its ability to improve water quality properties, including increasing DO and reducing temperature and TAN. In this study, an increment of DO and temperature, as well as reductions of TAN was observed. The DO, temperature, and TAN average for control after 24 hours were 9.94 mg/L (95%CI: 9.44, 10.4), 24.90C (95% CI: 24.8 to 25) and 2.58 mg/L (95%CI: 0.91, 4.25). Comparatively, DO, temperature, and TAN averages of nanobubble treatment group after 24 hours were 10.52 mg/L (95% CI: 9.86, 11.2), 24.60C (95%CI: 24.5, 24.7), and 2.35 mg/L (95% CI: 0.86, 3.84), respectively. Likewise, the use of nanobubble in PL10 hatchery can increase the DO by 6%, reduce the temperature by 2%, and reduce the TAN by 9%.

The DO increment under nanobubble treatment recorded in this study was comparable to other results obtained by Ebina et al. (2013) and Rahmawati et al. (2020). According to Ebina et al. (2013), nanobubble significantly increased the DO by four folds. The DO in control was 7.7 mg/L and in nanobubble treatment it increased four times to 31.7 mg/L. Similarly, Wu et al. (2019) reported DO increased almost by nine folds from 0.60 mg/L to over 5.00 mg/L, while Rahmawati et al. (2020) reported that after 40 days, the DO level with nanobubble treatment was 5 mg/L, while the control was 3 mg/L.

Besides increasing DO, nanobubble has been reported to remove water pollutants including reducing ammonia nitrogen, total nitrogen, TDS, and total phosphorus. The high zeta potential and a large specific surface area of nanobubble can assist in the removal of pollutant (Xia & Hu, 2018). These nanobubble characteristics create longer contact time between the bubbles and the suspended matter, thus improve the adhesion efficiency. The combination of nanobubble and coagulation technology can enhance the removal of pollutants in water. In Liu & Tang (2019) study, the reduction rate of ammonia nitrogen was reported to be equal to 80%. The TAN under nanobubble treatment was observed at 9% lower than control and this indicated that there was TAN removal. The ammonia removal was conducted through the oxidation process and nanobubble was proven to be rather effective in oxidizing ammonia (Khuntia, Majumder, & Ghosh, 2013).

Conclusion

The nanobubble technology used in the *P. vannamei* PL 10 hatchery was able to manage the water quality properties. To conclude, the nanobubble can increase DO level while simultaneously reduce the temperature and TAN. The DO in nanobubble treatments increased by 6%, the temperature decreased by 2%, and the TAN reduced by 9% after 24 hours. Further research should be conducted to measure physiology condition of reared *P. vannamei* using nanobubble.

Acknowledgement

This study was funded by PUTI UI 2020-2021 Grant Number 2026 funding scheme of Universitas Indonesia, and supported by Nano Center Indonesia. The nanobubble machinery was provided by Nanobubble Karya Indonesia Ltd., South Tangerang, Indonesia, and patented by Pusat Penelitian Fisika LIPI No. P00201903600. The authors would like to express our gratitude to Emmanuel Ramli and Agus Budiana for the onsite research opportunities. We would also like to thank the Nanobubble Karya Indonesia's team for their technical assistance.

References

- Cheng, W., Wang, L. U., & Chen, J. C. (2005). Effect of water temperature on the immune response of white shrimp *Litopenaeus vannamei* to *Vibrio alginolyticus*. *Aquaculture*, 250(3–4), 592–601. doi: 10.1016/j.aquaculture.2005.04.060
- Djunaedi, A., Susilo, H., & Sunaryo, S. (2016). Kualitas Air Media Pemeliharaan Benih Udang Windu (*Penaeus monodon* Fabricius) dengan Sistem Budidaya yang Berbeda. *Jurnal Kelautan Tropis*, 19(2), 171. doi: 10.14710/jkt.v19i2.846
- Ebina, K., Shi, K., Hirao, M., Hashimoto, J., Kawato, Y., Kaneshiro, S., ... Yoshikawa, H. (2013). Oxygen and Air Nanobubble Water Solution Promote the Growth of Plants, Fishes, and Mice. *PLoS ONE*, 8(6), 2–8. doi: 10.1371/journal.pone.0065339
- Galang, D. P., Ashari, A. K., Sulmatiwati, L., Mahasri, G., Prayogo, & Sari, L. A. (2019). The oxygen content and dissolved oxygen consumption level of white shrimp *Litopenaeus vannamei* in the nanobubble cultivation system. *IOP Conference Series: Earth and Environmental Science*, 236(1). doi: 10.1088/1755-1315/236/1/012014
- Khuntia, S., Majumder, S. K., & Ghosh, P. (2013). Removal of ammonia from water by ozone microbubbles. *Industrial and Engineering Chemistry Research*, 52(1), 318–326. doi: 10.1021/ie302212p
- Liu, C., & Tang, Y. (2019). Application research of micro and nano bubbles in water pollution control. *E3S Web of Conferences*, 136, 10–12. doi: 10.1051/e3sconf/201913606028
- Mahasri, G., Saskia, A., Apandi, P. S., Dewi, N. N., Rozi, & Usuman, N. M. (2018). Development of an aquaculture system using nanobubble technology for the optimization of dissolved oxygen in culture media for Nile tilapia (*Oreochromis niloticus*). *IOP Conference Series: Earth and Environmental Science*, 137(1). doi: 10.1088/1755-1315/137/1/012046
- Ohmori, M., Haruta, K., Kamimura, S., Koike, H., Uchida, T., & Takeyama, H. (2015). A Simple Method for Nanobubble Generation and Stability of the Bubbles. *Journal of Environmental Biotechnology*, 15(1), 41–44.
- Rahmawati, A. I., Saputra, R. N., Hidayatullah, A., Dwiarto, A., Junaedi, H., Cahyadi, D., ... Rochman, N. T. (2020). Enhancement of *Penaeus vannamei* shrimp growth using nanobubble in indoor raceway pond. *Aquaculture and Fisheries*, (December 2019). doi: 10.1016/j.aaf.2020.03.005
- Sahrijanna, A., & Sahabuddin. (2014). Kajian kualitas air pada budidaya udang vaname. *Prosiding Forum Inovasi Teknologi Akuakultur 2014*, 329–336.
- Supriatna, Marsoedi, Hariati, A. M., & Mahmudi, M. (2017). Dissolved oxygen models in intensive culture of whiteleg shrimp, *Litopenaeus vannamei*, in East Java, Indonesia. *AAFL Bioflux*, 10(4), 768–778.
- Tekile, A., Kim, I., & Lee, J. Y. (2016). Extent and persistence of dissolved oxygen enhancement using nanobubbles. *Environmental Engineering Research*, 21(4), 427–435. doi: 10.4491/eer.2016.028
- Temesgen, T., Bui, T. T., Han, M., Kim, T. il, & Park, H. (2017). Micro and nanobubble technologies as a new horizon for water-treatment techniques: A review. *Advances in Colloid and Interface Science*, 246, 40–51. doi: 10.1016/j.cis.2017.06.011
- Valencia-Castañeda, G., Frías-Espéricueta, M. G., Vanegas-Pérez, R. C., Pérez-Ramírez, J. A., Chávez-Sánchez, M. C., & Páez-Osuna, F. (2018). Acute Toxicity of Ammonia, Nitrite and Nitrate to Shrimp *Litopenaeus vannamei* Postlarvae in Low-Salinity Water. *Bulletin of Environmental Contamination and Toxicology*, 101(2), 229–234. doi: 10.1007/s00128-018-2355-z

- Vinatea, L., Gálvez, A. O., Venero, J., Leffler, J., & Browdy, C. (2009). Oxygen consumption of *Litopenaeus vannamei* juveniles in heterotrophic medium with zero water exchange. *Pesquisa Agropecuária Brasileira*, 44(5), 534–538. doi: 10.1590/s0100-204x2009000500014
- Vinatea, L., Muedas, W., & Arantes, R. (2011). The impact of oxygen consumption by the shrimp *Litopenaeus vannamei* according to body weight, temperature, salinity and stocking density on pond aeration: A simulation. *Acta Scientiarum - Biological Sciences*, 33(2), 125–132. doi: 10.4025/actasciobiolsci.v33i2.7018
- Wang Yangcai, Liu Youyu, S. Y. (2018). Primary application of nano-bubble technology in shrimps. *Fishery Modernization*, 45(2), 21–28.
- Wu, Y., Lin, H., Yin, W., Shao, S., Lv, S., & Hu, Y. (2019). Water quality and microbial community changes in an urban river after micro-nano bubble technology in situ treatment. *Water (Switzerland)*, 11(1). doi: 10.3390/w11010066
- Wyban, J., Walsh, W. A., & Godin, D. M. (1995). Temperature effects on growth, feeding rate and feed conversion of the Pacific white shrimp (*Penaeus vannamei*). *Aquaculture*, 138(1–4), 267–279. doi: 10.1016/0044-8486(95)00032-1
- Xia, Z., & Hu, L. (2018). Treatment of organics contaminated wastewater by ozone micro-nano-bubbles. *Water (Switzerland)*, 11(1). doi: 10.3390/w11010055
- Zhou, B. L., & Boyd, C. E. (2015). Ammonia nitrogen management in aquaculture ponds. (November), 1–6.